

IPCC's ritual on global warming

If the threat of global warming is serious (which cannot be denied), it deserves more seemly ways of making authoritative opinion public than that followed at last week's meeting at Maastricht.

So the greenhouse effect is real, then? That will be the first reaction of those who read of last week's meeting at Maastricht of the Intergovernmental Panel on Climate Change (IPCC), which is preparing its second assessment of the extent to which greenhouse gases in the atmosphere will affect the Earth's radiation balance. By all accounts (but see page 274), carbon dioxide has continued to accumulate, but only half as quickly as carbon dioxide is generated by the combustion of fuel. (The remainder is probably locked up in the biosphere, or dissolved in the oceans, temporarily or otherwise.) And while the concentration of methane in the atmosphere is increasing at a decelerating rate, IPCC says that, molecule for molecule, its effect on climate is greater than previously allowed. But interested readers (of whom there are in principle about 5 billion) will have to wait until the secretariat has taken account of last week's discussion, and until Cambridge University Press has turned the outcome into type, before they will be able to weight the quality of the discussion.

This is a rotten way to conduct international business, the more so because literally everyone in the world will eventually be affected by it. Last week's reports from Maastricht suggest that the goal of restraining emissions of carbon dioxide below those of 1990 (the European Union's collective goal) will be insufficient even to prevent a further doubling of carbon dioxide concentration in the atmosphere. That has been on the cards from the outset, but the apportionment of allowable emissions among the potential claimants on them will be a much more difficult task than the negotiation of the Convention on Climate Change at Rio de Janeiro two years ago, where it must have seemed to many that a mere signature could prevent climatic deterioration. If IPCC is serious (and there is no reason to believe otherwise), it should now be doing everything it can to make the further agreements that will be necessary winnable.

Communication by press release and "Executive Summary" (a euphemism for sound-bites directed at those who do not read) is no way in which to do that. What the world needs is a measured critical review of the literature on greenhouse gases and their effects on climate, perhaps covering the period since the last assessment in 1990. That is exactly how the UN Scientific Committee on Energetic Atomic Radiation (UNSCEAR) has conducted its business since its creation at the instance of the government of India in the 1950s. Such a framework is an entirely suitable vehicle for considered opinions on the significance of emerging

trends, and indeed is particularly well-suited to the consideration of the global warming problem, where uncertainties now extant are likely to be removed as the years pass. A useful format for IPCC's reports would be a listing of the continuing uncertainties and a periodic discussion of the extent to which they had been removed.

On this occasion, the press release put out from Maastricht declares that "the scientific consensus established in 1990 by the IPCC on climate science still holds". What does that mean? Certainly not that IPCC or its sponsoring agencies, the UN Environmental Programme and the World Meteorological Organization, were the first to define the global warming issue (which was almost the single-handed creation of Dr Roger Revelle at Harvard University). Unanimity? Nobody denies that carbon dioxide is a greenhouse gas, but argument persists in the research community about the effects on climate. To be persuasive, IPCC must show that it has given these issues the respectful considerations their origins command. Sadly, we shall not know for some time whether that essential obligation has been discharged. □

Discoveries for Africa

Africa deserves a big share of the pride in early hominid discoveries.

THE interest of the accounts on pages 306–312 and 330–333 of the latest australopithecine species to be recovered from Ethiopia is unlikely to be overlooked. With an age estimated at 4.4 million years, *Australopithecus ramidus* is almost a million years older than *A. afarensis* and that much closer to the probable divergence of the hominid line from that of the Great Apes, estimated by molecular cladists at about 4–6 million years ago. That means that the most conspicuous gap in the pre-human fossil record has been filled even though, as always, the need for further specimens to yield more detail will remain.

Interest and importance apart, it is important that a few temptations should be avoided. The similarity of *A. ramidus* with the chimpanzee, rather than the gorilla, is remarked on by the authors of the new discovery as well as by Dr Bernard Wood (see page 280). That will lead many to conclude that *Pan*, the chimpanzee, was the closest living relative to the early hominids. But that is inference only, absent a better understanding than at present of the course of evolution of



the apes themselves. By the same test, the attractive epithet of the 'missing link' had better be avoided until it is possible to answer with some clarity the question "With what?"

But one thing is clear. All the species of *Australopithecus* so far identified have come out of Africa, while the two African apes are the only plausible progenitors of the line. It is therefore excellent that the non-African members of the field expedition in Ethiopia have shouldered as a part of their obligation to their hosts to train a number of young people from Africa in the techniques of palaeoanthropology. Is it too much to hope that, in the long run, there will be free-standing expertise in this field not just in Ethiopia, but elsewhere in a continent from which too little good news comes? Mr Nelson Mandela might even take up the cause as a pan-African cause. □

Karl Popper is dead

Sir Karl Popper, who died a week ago, was a combatant on every intellectual battlefield he could find.

SIR Karl Popper's influence on science notwithstanding, his most important book was *The Open Society and its Enemies*, published in 1946. It is not an anti-communist tract as often represented, but a definition of the principles of liberality by which a community of people may foster its own well-being, and that of its children. That is what he will be most remembered for.

But Popper, almost single-handed, brought about a decisive change in the status of theoretical propositions in science that will be permanent, and which has done much to moderate the tendency towards dogmatism that is the bane of some professional opinions. Popper himself has explained how his arguments on this score grew from his abreaction against the logical positivists who, at the turn of the century, commanded his native Vienna's philosophy. But Ernst Mach, both a positivist and temporarily Einstein's inspiration, and Popper were not so far apart.

Popper's influence in science derives from the simple observation that the propositions described in science as 'laws' cannot be verified, but can be proved false by a single counter-example. From that it follows that even apparently durable propositions are repeatedly falsified. Thus was Newtonian mechanics in turn succeeded by special and general relativity, for example. The effect is that prudent scientists no longer proclaim that they have found the 'truth' about something, but merely that they have a better approximation to it. That is how it should be, in an open society.

True to his principles, Popper was catholic in his interests. As a philosopher, he dealt with everything. As a contributor to *Nature*, his more recent articles included a disputed proof that inductive probability is impossible (302, 687; 1983), a *gedanken* experiment to distinguish between the interpretations of quantum mechanics based on complementarity and that after which Einstein (and Popper) hankered and an advocacy of Wächterhäuser's theory of the origin of life on the surface of iron pyrites (344, 387; 1990). □

Genes and patent law

Over-zealous patenting of mutated genes could decelerate discoveries the law intends to encourage.

If our genes are our birthright, are they patentable? That is the question raised afresh by last week's announcement that the first of the genes linked with inheritable breast cancer, known as *BRCA1*, has now been identified and characterized (see pages 271 & 279). Under an agreement between the University of Utah (which gave us successively the first mechanical heart and cold fusion) and a company called Myriad, the identity of the gene will be the basis for a diagnostic test that will, then, be patented. Under present law, there is no reason why that should not be done. The question that arises is whether the law is sound.

On the face of things, patent law has worked excellently in this case. Although the link between the mutations of *BRCA1* so far identified and the occurrence of breast cancer in the families carrying it is far from clear, it is certain to be an invaluable tool in the search for other families in which early-onset breast cancer is apparently inherited. It should also serve as a means of genetic counselling for those at risk. In that sense, it is an illustration of the patent system at its best that entrepreneurs have ventured time and money on the search for a diagnostic test. Are these not exactly the circumstances in which they deserve patent protection for their efforts?

There are several grounds for doubting that simple proposition, not the least of which is that the hunt for these particular genes has occupied many other groups, mostly at academic institutions, for several years, many of whom have now been pipped at the winning-post. That spells wasted effort, to say the least of it. It probably also implies a new degree of deliberation in the way that academic research groups share information about their work with industrial enterprises. The time could come when research grant applications are routinely negotiated with potential industrial partners before being sent off to grant-making agencies. By creating new restraints, the result could be a deceleration of the pace of research in molecular genetics.

The consequences of that could be serious. Important though the new developments are in themselves, the big prize is that *BRCA1*, its relative *BRCA2* and the other predisposing genes yet to be recognized and mapped onto the human genome will in due course provide clues to the mechanism of sporadic breast cancer arising in families that do not carry mutated versions of the genes so far identified. Much the same is likely to be true throughout the world's several human genome projects, where the effort spent on the identification of genes is likely to prove only a small fraction of that required to work out their normal function in the tissues in which they are expressed. Yet that is where clues to the treatment and prophylaxis of disease are most likely to arise. There will be hopeless muddles if these projects are undertaken only after the groups concerned have first made a deal with a potential patent-holder. □

Breast cancer discovery sparks new debate on patenting human genes

Paris, Houston and London. The patenting of a gene for breast cancer susceptibility, whose discovery was announced last week by the US biotechnology company Myriad Genetics (see pages 270 and 279) has reopened the debate about how best to commercialize results from the human genome project — and whether patents should be granted on individual genes.

Mark Skolnick, from the University of Utah in Salt Lake City, headed the team which won the race to isolate the gene *BRCA1*, whose approximate location was first identified in 1990 by Mary-Claire King, a geneticist at The University of California at Berkeley. (The baton immediately passed to runners racing to isolate a second breast cancer gene, *BRCA2*, just localized by a team headed by UK researchers; *Science* will publish the papers on 7 October and 30 September, respectively.)

Some genome researchers are anxious, however, about the decision of Myriad Genetics, a company cofounded by Skolnick in 1991 (see below), to patent *BRCA1*. King, who describes Skolnick's isolation of *BRCA1* as "a lovely piece of work", attributes these worries to the fact that *BRCA1* is the first gene of potentially great importance for

testing large numbers of people.

Despite the passions it has aroused, the patenting of *BRCA1* may help to clarify the debate about whether human genes should be patented. This was sidetracked in 1991 by the bid — later withdrawn — by the US National Institutes of Health (NIH) to patent thousands of cDNA fragments.

The arguments in the Venter case centred on whether gene fragments whose function was unknown could be patented, and on whether NIH should potentially gain control over hundreds of products to which it had contributed little. In contrast, there is an emerging consensus that current patent law allows genes whose function are known — such as those for human interferon and relaxin — to be patented.

But the ethical debate continues. Noëlle Lenoir, president of the international bioethics committee set up last year by the United Nations Educational Scientific and Cultural Organization, says the committee has not yet taken a position on patenting genes.

But she says she believes it would fall outside the remit of her committee, which is mainly concerned with human rights issues raised by the human genome project. Patenting genes, she says, could be more a

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'Molecular mammography' will soon complement traditional screening.

question of scientific ethics than bioethics.

From one point of view, patenting human genes has less to do with patent law (or bioethics) than with whether it will encourage or hinder investment, and thus progress, in gene-based technologies. Public resistance to patenting genes also remains strong. But some dismiss this as emotional, pointing out that DNA sequences are molecules, like any other patentable chemical structure.

Nevertheless, as last week's announcement has revealed, many in the research community remain uncomfortable about patenting genes. Much of this discomfort stems from their belief that the human genome project should be a cooperative search for new knowledge, rather than a self-interested search for profits.

Mike Stratton one of the scientists from the Institute for Cancer Research in London who have localized the *BRCA2* gene, points out that all potentially useful genes are having patents taken out on them. "Many clinical geneticists are uncertain how positive it will be for the people concerned."

The recent explosion of commercial genome companies is already leading to demands for greater intellectual property protection as private funding outstrips the relatively small amount of state funding for genome research. Myriad — and others, such as Millennium (see *Nature* 368, 175; 1994) — point out that companies need returns on the massive investments needed to pay for heavy development costs, in particular to close the 'therapeutic gap' between the availability of diagnostics and subsequent therapeutics.

Stephen Friend, a researcher at the Cancer Center at Massachusetts General Hospital, says the isolation of the *BRCA1* gene "feels like a new era in terms of the power of industrial support, providing the opportunity that allowed a superb set of experiments to proceed very quickly." ▶

Myriad: spearheading a hunt for genes

Houston. Myriad Genetics Inc was founded in 1991 jointly by Mark H. Skolnick, professor of medical informatics at the University of Utah, and Walter Gilbert of Harvard University, the Nobel prizewinner who also helped to found Biogen, one of the first major biotechnology companies, in 1978.

Skolnick, who is vice-president of research at Myriad, says his time is split equally between Myriad and the university. He says that he formed the company, which is privately held with headquarters in Salt Lake City, Utah, because the university was not in a position to provide either the full scientific or financial resources needed to pursue "the common genes".

Myriad's initial plans are to focus on discovering genes linked to diseases such as cancer of the breast, colon, lung, and prostate, and heart disease. It is already pursuing the *BRCA2* gene, whose approximate location was also announced last week (the British researchers involved in the discovery say that, after the controversy surrounding patent rights, they have dropped plans to collaborate with Myriad on this project).

Two and a half years ago, Myriad agreed a worldwide exclusive licence to the gene

with the University of Utah. The company subsequently entered into sublicensing agreements with Eli Lilly & Co. of Indianapolis and Hybritech of San Diego, Lilly's wholly owned subsidiary. In return, both companies provided it with financial backing. With the *BRCA1* gene now identified, Myriad plans to collaborate with Lilly in developing new therapeutic agents for treating breast cancer and with Hybritech to develop diagnostic kits.

Part of Myriad's corporate strategy, as outlined in a company document, is that it plans to file 'composition of matter' patents on any genes discovered, and on the products (proteins) and mutations of those genes. It also plans to seek 'method of use' patents for the diagnostic or therapeutic use of those genes.

Meanwhile, in another illustration of the increasing interest of genetic engineering companies in genome technologies, Genentech announced last week that it has made an investment in Sequana Therapeutics of San Diego, California, in exchange for an undisclosed amount of equity. The relationship gives Genentech the rights to negotiate for licenses to those of Sequana's discoveries which its main research sponsors decide not to take up, **D. G.**

► Stratton also acknowledges that "it's a new ball-game", pointing out that the companies set up to hunt for new genes "have budgets with an extra zero on the end compared to those of most academic groups".

Some researchers argue that patenting genes will slow down scientific progress, and that those seeking patent protection will delay publication. But others argue that communication could be eased by faster patenting procedures.

Skolnick hopes people will not prejudge Myriad on its licensing policy. "We want to collaborate, and we're not going to slit our throats by cutting everybody off or by charging exorbitant amounts," he says. "As we develop a policy, I'm sure we'll test the waters, and if we get whacked good and hard then we'll hopefully refine the policy."

But it is in the European Union (EU) that the outlook for gene patents remains cloudiest. The European Parliament is opposing parts of a European Commission directive that would standardize national laws on patenting life. In its current draft form, the directive rules that parts of the human body, including genes, cannot be patented when they remain in the body.

After the Council of Ministers decided this week not to accept amendments proposed by the parliament, representatives of the two sides will now have to meet to try to agree a single text. But, rather than adopt a directive that explicitly banned patenting genes, ministers could well abandon the whole draft.

Declan Butler & Diane Gershon

Court rules cell lines are protected by law

Washington. In a precedent-setting decision, a court in Baltimore, Maryland, has required a researcher at the National Institutes of Health (NIH) in Bethesda, Maryland, to pay \$5,000 for deliberately destroying a cell line developed by co-workers in his laboratory.

After a five-day trial, Judge Peter Messitte ruled that Prince Kumar Arora, a researcher in the NIH's Laboratory of Neuroscience, destroyed cells developed by a colleague, Yochitatsu Sei, as part of an investigation of the impact of certain molecules on the brain.

Imposing both compensatory damages of \$400 and punitive damages of \$5,000, the court concluded that Arora had been motivated by jealousy. The ruling effectively enlarges the concept of 'conversion of property' to give protection to genetically engineered cell lines developed during the course of a research project.

The NIH, which participated as a civil party in the suit, had asked the court to accept the idea of 'conversion', reserved until now for circumstances in which one individual illegally appropriates the proceeds of property belonging to another. □

EPA rebuffs challenge to its assessment of dioxin data

Washington. With its latest study of health risks from dioxins nearly complete, the US Environmental Protection Agency (EPA) last week went out on a limb to say that dioxins probably cause cancer in humans. But the agency says it still needs additional data before making this conclusion definitive; and the case may never be closed entirely.

EPA has released a "public review draft" of its three-year reassessment of dioxin risks, the most exhaustive scientific review of any family of compounds ever undertaken by the agency. As expected, the agency is sticking with its 1985 assessment that dioxins are a proven animal carcinogen and a likely human carcinogen, even in trace amounts.

In addition, the EPA draft says that dioxins may have other effects on health, including reproductive problems and suppression of the immune system, although it is not yet clear at what dosage those effects occur in humans.

The report will not be finalized until September 1995, following a four-month public comment period, and a review by EPA's Science Advisory Board early next year. Meanwhile, the agency is calling for additional data from scientists, industry, government agencies and any other groups that might have new information on dioxin.

The draft report points out that there are large gaps in researchers' understanding of dioxin sources and the levels at which it exists in the environment and in food, the principal route of human exposure. EPA estimates that 95 per cent of dioxins come from incinerators that burn medical and municipal waste, but says there may also be unknown sources.

EPA initiated the reassessment in 1991, after some scientists had begun to question whether the risks from dioxin had been overstated. Research showing that TCDD, the most toxic of the dioxins, had first to bind with a receptor protein before causing any harm to cells implied that there might be a threshold before the binding occurred below which dioxin was not a problem.

EPA's models assume there is no such threshold, and therefore no 'safe' level of dioxin. Most European countries, as well as Canada, think this model is unrealistic, and have set their dioxin exposure limits much higher. But, after three years of reviewing the data, and conducting dose-response studies in its own laboratories and others, EPA still has not identified a threshold amount of dioxin that triggers a response.

Other incriminating studies were published during the three-year reassessment, including stronger epidemiological data link-

ing dioxin exposure to excessive cancer cases in Seveso, Italy, the site of an explosion in 1976 at a chemical plant. Other recent research has tied dioxin to endometriosis in rhesus monkeys and the suppression of the immune system in mice.

Much of the work for EPA has been spent staying abreast of these new findings. Lynn Goldman, the agency's assistant administrator for pesticides and toxics, says, "Every week there are new studies that are published. It's a challenge to keep up with the data."

Mindful of past criticism of its risk analysis methods, EPA has gone to great lengths during the dioxin reassessment to include participation of external scientists. Earlier drafts of the study went through three separate peer reviews, as well as a review by scientists in other federal agencies.

The authors or co-authors of several chapters were in fact scientists outside EPA. "This has been a new approach for the agency, to really make this an inclusive process," says Linda Birnbaum, director of the agency's Environmental Toxicology Division in Research Triangle Park, North Carolina.

Birnbaum says that providing definitive proof that dioxin causes cancer in humans could be a very tough proposition. "We need unequivocal human data, and that's very difficult to get."

One of the problems with dioxin epidemiology, says Birnbaum, is that these chemicals do not cause a unique kind of tumour that is readily identifiable. As a result, dioxin effects would not stand out from those of any other carcinogen. "We're looking at relatively small incidences over very large background incidences," she says.

Despite its contention that even minute amounts of dioxin pose a health risk, EPA is keen to avoid appearing alarmist. There is relatively little dioxin in the environment — only 30 pounds in the entire United States, by one estimate. As a result, says Goldman, even though dioxins accumulate in fatty tissue and show up in beef, dairy products and fish, the amounts are so small that the benefits of a healthy diet far outweigh the risks from dioxin.

Indeed, EPA says it will not take any new regulatory action until the results of the reassessment have been finalized next year. Considering that it has already proposed new rules cutting dioxin emissions from pulp and paper mills and municipal waste incinerators, and that it expects to propose new rules on medical waste incinerators next February, there may not be much dioxin around by the time the last bit of scientific evidence is in.

Tony Reichhardt

US agencies 'agree earmarks by telephone'

Washington. A committee of the US House of Representatives is due to hear accusations this week that some federal agencies are so eager to please their congressional paymasters that they accept instructions over the telephone to 'earmark' science money for pet projects in their constituencies.

But at least one university president — John Silber of Boston University — is expected to support earmarking as a way of funding new laboratories. Without it, he says, the United States would never have constructed a whole stream of world-famous facilities, including Fermilab, the nuclear weapons laboratories and the Jet Propulsion Laboratory in California.

Furthermore, with both congressmen and senators preparing for November's mid-term elections, the debate takes place at a time when earmarking seems about to rebound from last year's dip. The election is likely to be accompanied by much talk of the need for fiscal responsibility. But a possible power switch from Democrats to Republicans in the Senate — and perhaps even the House — would mean new committee chairmen, and thus a new set of universities well placed geographically to benefit from political largesse.

In the US system, budgets and priorities are prepared by federal agencies in cooperation with the administration. They are then sent to Congress for approval. Those who do this have always 'earmarked' a few dollars for their pet projects. But, over the past ten years, the scale of the practice has grown drastically, leading critics such as George Brown (Democrat, California), chairman of the House Science, Space and Technology Committee, to allege that it is undermining the process by which agencies are supposed to allocate funds on the basis of merit.

At a series of hearings this week, Brown will question officials of the Environmental Protection Agency (EPA) about telephone calls it is said to have received from congressional committee staff members. He is planning to use the hearings to press the Clinton administration to issue an executive order reminding agencies that they are not obliged to fund projects that appropriation committees ask for in the report language attached to budget bills.

Brown will also question the presidents of several universities which have lobbied Congress successfully for earmarked funds. At least one — Silber — will offer a vigorous defence of earmarking as helping science departments in small institutions to compete with the handful of leading universities that win the bulk of federal funding for peer-reviewed science.

Universities large and small have privately expressed misgivings about the timing of the Brown hearings, which coincide with the conference between the House and

Senate to finalize the defence budget bill. The House side, led by John Murtha (Democrat, Pennsylvania), has proposed cutting by half the \$1.8 billion spent by the Department of Defense on university research. But university officials say the final budget figures were expected to be agreed before the hearings, with university research cut by about \$180 million.

The hearings take place soon after another budget bill with a strong science con-



tent, covering agencies such as the National Science Foundation and the National Aeronautics and Space Administration, was passed by Congress with an unexpectedly large number of earmarks attached, with a total value of \$290 million. This includes \$70 million for universities, compared to \$8 million last year. A move by Brown on the floor of the House to eliminate these earmarks failed last week by nine votes.

At this week's hearing, Martha Krebs, director of research at the Department of Energy (DoE), was expected to defend her department's decision to divert funds away from the administration's stated research priorities — specifically, the Human Genome Project — to pay for earmarked

projects apparently unrelated to the department's missions.

The committee was also planning to question M. R. C. Greenwood of the White House Office of Science and Technology Policy about how far the administration is prepared to resist pressure from Congress to quietly fund earmarks. Greenwood is likely to restate the administration's opposition to earmarking, but her testimony will fall short of promising the executive order which Brown seeks.

It will then move to the Department of Defense (DoD). John Deutch, the deputy defence secretary — a firm opponent of earmarking — is likely to confirm the corrosive effects of the practice. But congressmen will want to know why so little has been done at the Pentagon to discourage it since Deutch's arrival last year.

Silber is set to clash directly with Brown when he gives evidence defending the recent earmarking of \$28 million of DoD money to build a photonics research centre at Boston University. Silber describes as "sheer hypocrisy" the way in which leading research universities have sided with Brown when they "all have had earmarked funds in the past".

According to Silber, Boston University has established highly successful departments — including the sixth best physics department in the United States, in terms of papers cited — through obtaining earmarked funds to build facilities. "The Congress has got a hell of a bang for their buck" through these earmarks, he says.

Silber does not deny using lobbyists to win earmarks, although he vehemently denies that earmarking is biased by the allocation of seats on congressional appropriations committees. He concedes that the Boston photonics centre, which will explore the science of optical signals, was not in the DoD budget request. "But it was a DoD priority, and the DoD was very pleased to have it," he says.

Colin Macilwain

UN meeting backs contraceptive research

London. Attempts by the Vatican to dilute key phrases in the programme of action agreed at the United Nations conference on population, which ended in Cairo last week, failed to alter significantly the thrust of the 20-year plan.

The Vatican's action meant that delegates at the main session spent much of the ten-day conference discussing references to abortion. But relatively few changes were made to the wording of the final document — including sections asking for more funds to be spent on contraceptive research (see *Nature* 370, 498; 1994).

No figure was specified by the meeting.

But the need for new male methods and female barrier methods that protect against sexually transmitted disease was emphasized to the conference by Prakash Tandon, former president of the Indian National Science Academy.

Tandon was representing the Inter-Academies Panel on Population Development set up last year by most of the world's science academies. "Scientists are increasingly concerned with the decreasing [support for] research and development in the general field of population dynamics, and specifically in the field of contraceptive research," says Tandon.

Maggie Verrall

Climate group rejects criticism of warnings

London. The Intergovernmental Panel on Climate Change (IPCC) has reaffirmed its earlier conclusion that global carbon dioxide emissions must be reduced if the concentration of greenhouse gases in the atmosphere is to stabilize at levels that prevent 'dangerous' climate change.

In doing so, the panel has rejected claims by critics that its conclusions fail to take sufficient account of the implications of continuing uncertainties about mechanisms affecting climate change — for example, the newly acknowledged role of sulphate aerosols in cooling the Earth by reflecting energy from the Sun, or the full impact on the climate of the carbon cycle.

The impact of aerosols, for example, has been used to explain discrepancies between model predictions of warming in the Northern Hemisphere and the lower limits on observed temperature changes. But the report, containing new estimates of the effects of sulphate aerosols, warns that these are highly localized and "cannot be seen as a simple offset to greenhouse gas forcing".

The conclusions are contained in the latest special report from the IPCC's Working Group I, which were approved last week at a plenary session of the working group held in Maastricht in the Netherlands. The group is responsible for providing scientific information relevant to the Climate Change Convention signed at the Earth Summit in Rio de Janeiro in June 1992.

The IPCC is already drawing up a Second Assessment Report, but this will not be published until November 1995, after the first conference of parties in March in Berlin. At that meeting, countries that have ratified the convention will decide whether it should be strengthened.

At present, the treaty says that signatory countries should aim to restore emissions of carbon dioxide to 1990 levels by the year 2000, and eventually to stabilize greenhouse gas emissions at levels that prevent 'dangerous' climate change.

Since 1992, however, there has been continuing disagreement over the interpretation of the wording of the treaty, which some countries argue does not bind them to stabilize carbon dioxide emissions at 1990 levels after the year 2000. Many industrialized countries feel that even this commitment is not strong enough, but no consensus has yet emerged on what should be done.

At the tenth meeting last month in Geneva of the International Negotiating Committee (INC), the body made up of representatives of the signatory nations, negotiators rejected a German proposal under which industrialized nations would cut their emissions by a specified percentage before a set target date.

Germany, which will be the host country next year in Berlin, is still considering tabling such a proposal for discussion. Last week Klaus Töpfer, the German minister of the environment, told the federal parliament that he was contemplating proposing such a protocol with "like-minded countries", which could include Denmark and the Netherlands.

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Emission controls are still 'essential'.

Töpfer says he has not yet decided whether this will be for a new protocol to the treaty. But last week the European Parliament proposed that the European Union take a lead in putting forward a proposal that convention signatories commit themselves to reducing carbon dioxide emission by 20 per cent by 2005.

The new IPCC report says that, according to various carbon cycle models, stabilizing atmospheric levels of carbon dioxide at less than 750 parts per million by volume (p.p.m.v.), compared to pre-industrial levels of 280 p.p.m.v. and current levels of 360 p.p.m.v., would demand a drop in emissions to below 1990 levels.

In addition, the report provides revised assessments of the global warming potentials (GWP) of other greenhouse gases such as methane — most GWPs have been revised upwards by between 10 and 35 per cent — as well as the improved estimate of the cooling effect of sulphate aerosols.

The main function of the Maastricht meeting was to agree on the wording of the

executive and policy-makers summaries, which, together with supporting scientific materials, make up the Special Report. But, the meeting was not immune from pressure from representatives of nongovernmental organizations (NGOs).

Representatives of the fossil-fuel industry, for example, argued for the exclusion from both the executive and policy-makers summaries of calculations of the level of carbon dioxide emissions corresponding to a range of atmospheric stabilization scenarios, saying that the IPCC was not mandated to make such calculations. This suggestion was rejected, with the working group's chairman, Sir John Houghton, the former director of Britain's Meteorological Office, insisting that the IPCC had to be free to explore questions that it feels are relevant in order to maintain its scientific integrity and independence.

Others criticized the implications of the painstaking peer-review process used in producing the latest report. "It's consensus science, it runs the risk of being slightly behind the times," says Patrick J. Michaels, of the University of Virginia, the state climatologist for Virginia. "That's the nature of a consensus document."

Nevertheless — and despite the fact that many of the scientific data in the report have already been published elsewhere — Keith Shine, lecturer in meteorology at the University of Reading and lead author of one of the five chapters, describes the process of compiling the report as being "unquestionably valuable" in bringing all relevant information together in a single document.

The report also boosts the use of computer simulations in assessing the impact on the climate of particular events such as the eruption of Mount Pinatubo in June 1991. The eruption resulted in a large but short-lived increase in aerosols in the stratosphere. This in turn caused an observed surface cooling of about 0.4 °C, while model simulations predicted a global mean cooling of 0.4 to 0.6 °C; Shine describes such results as "encouraging".

Maggie Verrall

Underwater thumps baffle ocean scientists

San Francisco. Scientists based on the central California coast are trying to identify the origin of a mysterious underwater sound that disturbed surfers and divers for three weeks — and then just as mysteriously disappeared.

The sound, made up of thumps occurring at 10-second intervals, was compared by one diver to five or six giant bongo drums going off simultaneously. Most experts have concluded that it was of human origin. John Sanders of the Naval Postgraduate School in Monterey says that the school's scientists are confident that the sound was not pro-

duced by any naval activities, although they would not say if any navy submarines were in the area. The Scripps Oceanographic Institute denied that the noises were related to their controversial global warming tests, due to start in the area next year.

Khosrow Lashkari of the Monterey Bay Aquarium Research Institute suggests that the sound might have been the mating call of a large fish, such as a grouper. But, unless the sound returns, it is unlikely that the mystery will ever be cleared up.

Joel N. Shurkin

UK introduces 'research levy' on health care spending

London. Britain's Department of Health is to introduce a radical new system for funding health-related research, based on the distribution of a 'research levy' — currently estimated at just over one per cent — raised on money allocated to local health authorities for providing health care.

The estimated £340 million spent on research and development (R&D) every year by Britain's National Health Service (NHS) is distributed in a variety of ways, including lump sum grants allocated to teaching hospitals through the Service Increment for Teaching and Research (SIFTR).

The government is worried that there is often little accountability for the way the money is



Culyer: arrangements 'in need of change'.

spent. Others are increasingly concerned that research funding is being squeezed out by the short-term approach of the 'internal market' imposed on the NHS by successive Conservative administrations (see *Nature* 369, 514; 1994).

The new approach seeks to address both concerns through a system of 'managed competition' for research funds. Integrating all health research spending into a single funding stream will, says the government, help to ensure that funds are not diverted to other purposes. It will also require clinicians seeking backing for research projects to accept tighter scrutiny of both the scientific validity of proposals and the potential value of (and cost of applying) their results.

"This is an important new way of allocating our R&D funds," says Michael Peckham, chief scientist at the Department of Health. "I see it as a real prototype of the way in which other countries could organize their [health-related] research by the side of their health systems, and not snarled up in them."

The reforms are based on the recommendations of a task force set up by the Department of Health to look at the support of R&D in the NHS, and chaired by Anthony Culyer, professor of medical economics at the University of York.

The task force's report, based on written submissions from almost 200 institutions and professional organizations, as well as the work of three subgroups, paints a highly critical picture, reinforcing claims that even high quality research (for example, large-scale clinical trials) is threatened by recent NHS reforms.

In line with the government's view that

responsibility for spending decisions should be devolved to local authorities acting as the 'purchasers' of health care, some of those consulted proposed that research spending should be treated in the same way.

But the Culyer committee rejected such arguments, pointing out that research is carried out for "the common good" of the NHS. It suggested the introduction of a "common services levy" on all purchasers of health care, claiming that this would "symbolize common ownership of spending plans".

In releasing the committee's report last week, the Secretary of Health, Mrs Virginia Bottomley, said that the government had accepted its recommendation of uniting all NHS research spending into a single funding stream in order to secure "better targeting, management and accountability". Such spending represents about 1.1 per cent of the NHS's total budget, although the government has already committed itself to reaching an eventual target of 1.5 per cent.

Detailed discussions will soon begin between the department and the various bodies carrying out health-related research — ranging from university teaching hospitals to pharmaceutical companies that use NHS facilities for clinical trials of new drugs — on how the new system will be put into effect, and what interim arrangements are needed to ensure a smooth transition.

The negotiations will not be easy, particularly as some are unlikely to welcome the new arrangements. University teaching hospitals, for example, had hoped to retain their control over funds through the SIFTR arrangements.

At the same time, the government is planning to discuss with Britain's three higher education funding councils ways in which the grading exercises already being applied to university departments can be extended to the research activities of all teaching hospitals.

Although medical charities — as well as the Medical Research Council (see right) — have welcomed the extent to which the government appears to have adopted the main thrust of the Culyer report, namely that research funds need special protection within the new-style NHS, both are reserving their final judgement until they have seen how the new system is put into practice.

But Peckham's apparent success in persuading his political bosses that research spending requires protection within the NHS's market economy is said to have the backing of research managers in the pharmaceutical industry. These point out that the government's commitment to a centralized research strategy is similar to that of many of their own companies.

David Dickson

Health service to limit support for MRC-led trials?

London. Proposed changes in the way in which the British government finances health-related research (see left) will open a new chapter in the somewhat bumpy relationship between the National Health Service (NHS) and the Medical Research Council (MRC), the UK's main source of funds for basic biomedical research.

In particular, according to Michael Peckham, formerly director of the Royal Postgraduate Medical School and now the Department of Health's chief scientist, the NHS will be less willing than in the past to offer itself automatically as a test-bed for trials of techniques based on new discoveries made in MRC laboratories.

Under a 'concordat' signed with the MRC in 1992, the health departments promise to provide "the necessary service support for that research supported by MRC funds". But the task force chaired by Anthony Culyer says that this commitment is "too open-ended", as the NHS can devote only a finite sum to providing support for research and development (R&D) projects. "It should not be obliged to support new MRC R&D if it has good reason to believe that it has a better use for its money," the task force says.

Peckham describes the concordat as "in general a great success", but admits that "there have been problems". If a new trial is proposed on the basis of promising scientific results, he says, "we have to be able to ask whether it addresses what we consider to be an important problem, how much it is going to cost, and the likely benefit to the NHS," he says.

MRC officials have in general welcomed the planned new funding system, but are wary both of the likely impact of the extra administrative demands (for example for more peer review of proposals) and of pressures on NHS funding which could limit opportunities for long-term research projects into promising new treatments.

The MRC concordat, itself the result of a debate initiated by the Rothschild Report of 1972 over the role of the MRC as a 'contractor' meeting the needs of the health service as a 'customer', is due for renewal next year. Both sides accept the need for rewording, but negotiations may not be straightforward.

Peckham argues that "there needs to be a realistic understanding of what the NHS can sustain". The MRC wants to avoid a situation in which health departments become pre-occupied with meeting short-term goals at the price of long-term investment.

D. D.

Japan builds on global lead in nanotechnology

Tokyo. Despite the impact of the recession on overall research spending in Japan, one area in which efforts are continuing to expand rapidly is quantum science and technology, the study and application of nanoscale phenomena. Two reasons behind this enthusiasm are Japan's mastery of device fabrication — and its success in the commercial exploitation of the results.

In the West, thin-film growth techniques such as molecular beam epitaxy and metal-organic chemical vapour deposition remain exotic laboratory procedures. But in Japan, companies such as Fujitsu and Sony have already moved to the production line, for example to make quantum-well semiconductor lasers for compact disc player pickups and high electron mobility transistors for amplifiers for consumer satellite dishes.

Keen to build on this success, the Ministry of International Trade and Industry (MITI) earlier this month put in a bid for funds with the government that would more than double support for its new Ångström Technology Partnership project from US\$10 million to US\$22.4 million next year.

Two other government agencies are also pouring money into such research, and the private sector continues to back work on nanoscale electronics and photonics.

The Ångström project began in April 1993, and will run for ten years at an overall cost of \$250 million. The project differs from previous large-scale MITI initiatives in that it includes many researchers from universities, who come under the jurisdiction of the Ministry of Education, Science

and Culture (MESC), reflecting MITI's desire to stimulate a more open government research system (see *Nature* 371, 3; 1994).

Another difference is that, of 30 corporate participants, six are non-Japanese companies, five based in the United States and one (Samsung Electronics) in South Korea.

The extra money will help to pay for new equipment at the Joint Research Center for Atom Technology, the project's base in Tsukuba. This includes two supercomputers: a conventional vector processor, made by Fujitsu, and a massively parallel CM5E made by Thinking Machines of the United States.

At present, most research focuses on fundamental rather than applied issues. In particular, scientists are looking at ways of confining electrons and photons to one and zero dimensions in devices such as quantum wires and dots. To produce such devices, they need to develop techniques for fabricating structures whose smallest dimension is less than 10 nanometres.

At Tokyo University's Institute of Industrial Science, for example, Yasuhiko Arakawa has grown gallium arsenide quantum wires less than 10 nm wide, and quantum dots as small as 7 nm. His ultimate goal is to build single-electron devices.

Arakawa has had little difficulty with funding. From 1987 until March this year, his group was sponsored by MESC and private companies. Now, and up to 1997, government funding comes from a successor project, Quantum Coherent Electronics.

Arakawa's former mentor, Hiroyuki

Sakaki, is also about to embark on a second quantum device research project. For the next five years, the Research & Development Corporation of Japan — an offshoot of Japan's Science and Technology Agency — will fund Quantum Transition, a collaborative project between Sakaki's group at Tokyo University and the Center for Quantized Electronic Structures (QUEST) at the University of California, Santa Barbara.

JRDC's first foray into international collaborations was a programme called Atom Arrangement-Design and Control for New Materials, which began in 1990. This five-year, £5-million project brings together Japanese researchers with counterparts at the universities of Cambridge and London.

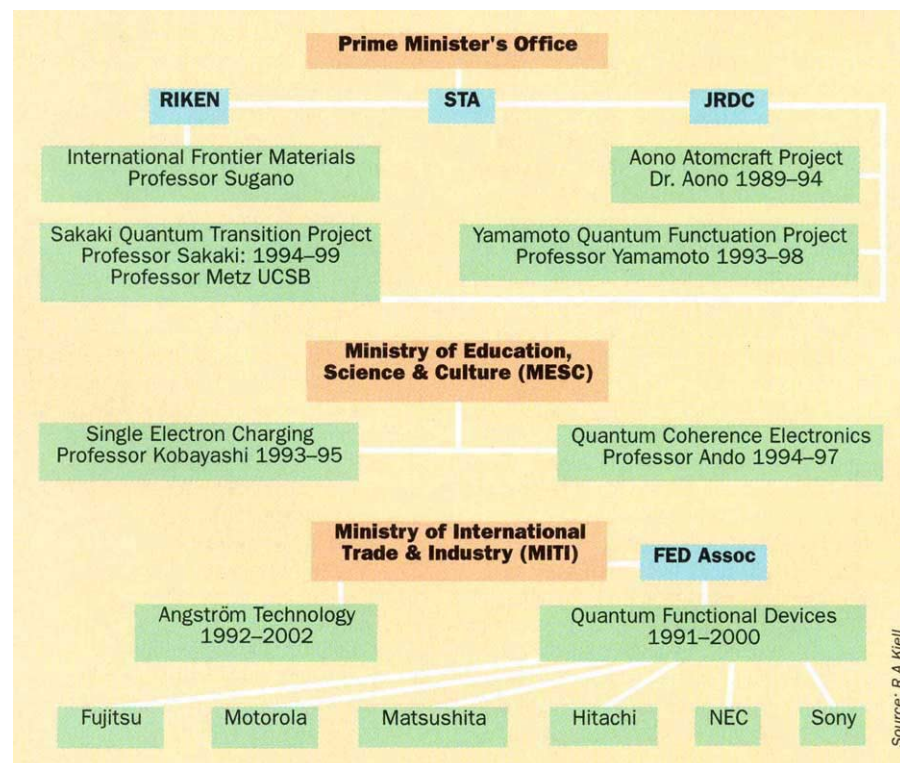
A third international JRDC quantum initiative is Yamamoto Quantum Fluctuation, a five-year, \$17-million ERATO project that began last year. Its leader is Yoshihisa Yamamoto, a laser physicist from Nippon Telegraph & Telephone (NTT) who is now a professor of electrical engineering at Stanford University in California. The project combines talents from Yamamoto's former and current research groups.

According to Seigo Tarucha, a supervisor at NTT's Basic Research Laboratories in Atsugi, about a hundred of the company's researchers are working on quantum phenomena. The emphasis is on techniques for epitaxial growth and device fabrication.

Across the road at Fujitsu's Atsugi laboratory, Naoki Yokoyama and his group have been working on resonant tunnelling transistors for almost ten years. Their ultimate aim is to produce extremely high density devices (in the 10 gigabit class).

Fujitsu's work is the furthest along of six investigations into new device concepts supported under MITI's Quantum Functional Devices project, which runs from 1991 to 2000. Other participants include Hitachi, Matsushita, NEC and Sony. One surprise is the participation of researchers at the laboratory in Phoenix, Arizona, of an American company, Motorola. Motorola is one of the last non-Japanese companies still active in quantum device research. In the United States, IBM and Bellcore have disbanded their formerly world-leading compound semiconductor groups; and in Europe, where Philips had the main research group, activity outside universities has largely ceased.

In contrast, Japanese companies are continuing to work in the field. Hitachi, for example, has between 70 and 80 researchers for work on quantum topics. Ten are currently working at a laboratory in Cambridge, England, but most are based at the company's Central Research Laboratories in Tokyo. The emphasis, however, is shifting from basic to applied research. "For Japanese industry, it is difficult to carry out basic research because of the recession," says ▶



Source: R A Kiehl

► Eiichi Maruyama, the former Hitachi research manager who heads the MITI Ångström Technology Partnership. That is why, Maruyama claims, companies welcome MITI's initiative.

Some Japanese researchers, however, are concerned about the large amounts of government funding going into the field. "The presence of so many projects is not a good sign," says Hiroyuki Sakaki. "Particularly for younger researchers, it may be more intelligent to look for some newer subject—one that will attract people in ten years' time."

Bob Johnstone

Latin America pleads for more technology

São Paulo. The heads of state and government of Latin American and Caribbean countries last week issued a joint plea to industrialized countries not to impose obstacles on the transfer of the technologies they need to modernize their economies and adjust to the pressures of international competition.

They also said, in addition to more access to Western technologies, they would like to participate in the development of new technology as "producers and consumers" of knowledge. But, although discussing the prospects for greater regional cooperation in science and technology, no new measures were agreed to. The only concrete result of such diplomatic promises is an Argentinian-Brazilian biotechnology centre created in the 1980s.

The heads of state were in Rio de Janeiro for the eighth meeting of the so-called Rio Group, and will present a document listing their conclusions to the American Summit in Miami next December. Among other things, the document says that "national and international controls for the transfer of dual technologies [those with both civilian and military applications] should not hinder the access to goods and advanced technologies of pacific use with the aim of development".

The heads of state point out that their region was the first to commit itself, through the Treaty of Tlatelolco, to remaining free of nuclear and other weapons of mass destruction. But the document is not intended to refer only to the uses of nuclear energy. Brazil, for example, feels that it has been treated unfairly over its Satellite Vehicle Launcher, which the United States fears may be converted into a ballistic missile.

A boycott by Western nations has meant that Brazil has turned to China for help with the development of satellites. And last week (15 September) Brazil signed a nuclear energy agreement with Russia that may lead to Russian assistance for Brazilian reactors.

Ricardo Bonalume Neto

● The former head of India's Atomic Energy Commission, M. R. Sirinivana, last week claimed that India possessed nuclear weapons, which were being kept as a "protection" against Pakistan.

Pasteur Institute climbs out of rags and into riches

Paris. Next year, the centenary of the death of Louis Pasteur, has been designated the "year of Pasteur" by UNESCO. It comes just as an internal review of the activities of the Pasteur Institute in Paris shows remarkable growth over the past six years, due mainly to large increases in its income from patents and commercial services, and legacies and donations.

Both trends would have pleased Pasteur himself, who considered applied and fundamental research as equally important, "linked as a fruit to its tree". Pasteur also began the institute's tradition of fund raising; it was founded in 1887 with FF2 million—around FF50 million (US\$9.4 million) at today's values—raised in this way.

The institute's budget has grown from FF550 million in 1988 to FF860 million last year a growth of 55 per cent in five years. According to the review, income from industry grew from FF70 million to FF160 million over this period, and from commercial services such as the preparation of monoclonal antibodies from FF53 million to FF70 million. As a result, their combined share of the total budget has increased from 20.5 to 26 per cent.

Legacies and donations, the Pasteur's other traditional source of income, increased from 18.8 to 27.2 per cent of the total budget. Legacies alone accounted for FF190 million in 1993; one donor, the British company Virgin, raised FF12 million for AIDS research at the institute through the sale of a compact disc recorded by a number of prominent singers.

As a result of the growth in Pasteur's own resources, even though government funding increased by 10 per cent between 1988 and 1993, its share of the total budget has dropped from 47 to 36 per cent. The new figures therefore represent a turnaround in Pasteur's long struggle to generate income and regain some of the independence it lost in 1965 when a financial crisis forced it to turn to the state for support.

At the time, Général Charles De Gaulle, the then French president, did not hesitate to help an institute that he once called "as untouchable as the Eiffel Tower". But his government made its support conditional on Pasteur separating its research from its commercial and industrial activities.

Pasteur's subsequent transition from a cottage industry into a one responsible for joint ventures with international companies has not been easy. In 1972, for example, it created Institut Pasteur Production (IPP). Sanofi, a subsidiary of the French oil company Elf, took a 35 per cent stake in IPP in 1976, but the company was still losing money in 1984. Profitability came only after Pasteur

handed over control of its vaccines and serums business to Institut Mérieux (a subsidiary of the Rhône-Poulenc group), and of its diagnostics business to Sanofi.

Pasteur-Mérieux Vaccines and Serums is now the world leader in the \$2-billion vaccine market, with three-quarters of its sales outside France (last year the company distributed a billion doses of vaccines). Pasteur-Sanofi Diagnostics ranks fifteenth in the world market, with sales of FF1.4 billion last year.

Much of Pasteur's recent success has come from its aggressive patenting policy. Income has tripled since 1988 and risen from 9 to 18 per cent of the total budget. The institute now has 254 patents, and gives a



The new FF 110 million scientific information centre which is being opened at the Institut Pasteur in Paris next week by Edoard Balladur, the French prime minister. Financed from a FF270 million legacy of jewels from the Duchess of Windsor, the centre will hold the 30,000 books and volumes of 2,500 journals of the existing library, one of the finest collections in microbiology.

share of royalties to individual researchers, although it recently fixed an annual ceiling of FF300,000 to prevent some researchers earning too much more than others.

All of these results have come as a boost to the standing of Maxine Schwartz, who was reelected earlier this year as director of the institute for a second six-year term. The internal review covered his first term of office, and during this period the institute also expanded and reoriented its research activities.

Concrete evidence of this growth includes a large new building for research on AIDS and retroviruses, the most visible evidence of the institute's massive investment in an area that involves a tenth of its researchers and its budget. Next week will see the opening of a FF110-million high-technology scientific information centre which symbolizes the institute's desire to increase its attractiveness to both French and foreign scientists.

Declan Butler

Problems of Indian science

SIR — Writing on "Science in India", (*Nature* 366, 611–626; 1993), John Maddox begins by saying that "never can there have been such a determination to attain improvement by science and technology as there has been in India since 1947, but success is sadly still long way off". He mentions achievements in fields such as space, atomic energy, radioastronomy and communications, but these activities do not touch the life of the common man.

The basic problem of development in India has been the decision-making process in Delhi, procrastination and secrecy. Many scientists/technologists in positions of authority have behaved like seasoned bureaucrats in shirking responsibilities and scuttling proposals.

When I was at the National Geophysical Research Institute, I was aware of specific instances where decisions were never taken on important proposals such as water resource development; the ECAFE (now ESCAP) proposal for joint offshore geophysical surveys in India's economic zone; airborne geophysical mapping for minerals, oil and ground water; a UNESCO proposal for a seismological network and a training and research centre in India; and detailed studies for earthquake hazard evaluation of Koyna and adjoining areas of Maharashtra.

A proposal was made for an integrated micro-level study that included all aspects of human life and its environment; it would have been implemented through a district-level task force involving the principal scientific agencies on the one hand and the state government agencies, voluntary organizations and local people on the other. But even though pilot projects in two or three districts were approved in January 1986 by the then prime minister, Rajiv Gandhi, it has still not seen the light of the day.

Science and technology have primarily helped only the 'rich' India. Even the basic necessities of life such as clean drinking water, hygienic living conditions and two square meals a day are not available to most of the population in rural India and those living in "the tin and cardboard shanty-towns on the outskirts of the major cities".

Maddox suggests that "the better strategy, for those for whom development is an urgent need, would be to exploit the willingness of the technical community elsewhere to assist". It may be an idea worth considering, but does India need foreign assistance? Every Indian prime minister since Pandit Nehru has urged scientists to make science and technology relevant to the needs of the people. Thousands of educated young people are unemployed, laboratories have rural technologies on the shelf and the present

prime minister, P.V. Narasimha Rao, has made available R30,000 crores (US\$10 billion) for rural development.

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SIR — In the discussion of misconduct in research, attention has centred on questions such as authorship and the influence of competition for research funds. I believe that the problem is much wider, and has its roots in the now almost universal separation of teaching and research. With the increasing concentration of research in dedicated laboratories and institutes, and the shift from basic to applied research, full-time researchers and research managers have developed a novel attitude towards research which they are able to force on members of the scientific community still working at teaching institutions. One result, of course, is the decline in the numbers of able people emerging from the classrooms.

The problem is especially serious in developing countries. In India, for example, where scientific misconduct goes to the roots of the academic system, a large part of the trouble is that crucial decisions about support for and organization of laboratory work are made by inactive and often elderly scientists. It has become the convention that to speak about scientific misconduct is considered to be an even greater evil than misconduct itself. It is high time that uniform standards in research and teaching were developed and applied globally.

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Self-help in Kerala

SIR — The elimination of the dread disease lymphatic filariasis, commonly known as elephantiasis, may soon become a reality in the state of Kerala where it has been endemic for the past century.

Filariasis, which is transmitted by the bite of mosquitoes, is known as *Manthurogam* or *Anakalu* in the vernacular language of Kerala. It is prevalent not only in Kerala but also throughout most of India. In India the disease is caused by two related parasites, *Wuchereria bancrofti*, which has a country-wide distribution, and *Brugia malayi*, which is mainly

restricted to Kerala. It is estimated that about 367 million people in India live in areas exposed to the risk of acquiring this infection.

Approximately 19 million people suffer from chronic forms of lymphatic filariasis and another 25 million are asymptomatic carriers. Though it does not kill, it can maim and deform. It also has an immense socio-economic impact on the relatives of victims because of the stigma attached to it. The victims and their families are often shunned by society when they try to establish matrimonial or other relationships. As the disease is not fatal, its control receives only a low priority for funding both at central and state government levels. Nevertheless, there is a ray of hope, as witnessed in Chertala, a small town in Kerala, where a people's movement, FILCO, has achieved what the government could not. This has been made possible with the help of a research team from the Vector Control Research Centre of the Indian Council of Medical Research.

This is the first experiment in India where the motivation and involvement of the community through social and economic developmental activities has contributed to near-elimination of transmission of the disease. This experiment may be the precursor of similar programmes elsewhere.

FILCO, which, has already done a great deal in motivating the community to control the vectors of disease, has now launched the sale of salt medicated with DEC (diethylcarbamazine citrate) in the hope of eliminating the remaining traces of infection in this community. DEC is a safe and effective drug widely used for the prevention and treatment of filariasis. In a country such as India, with inadequate funds for sustained vector control and lack of effective governmental machinery for conventional drug distribution, DEC medicated salt seems to be the only effective method of eliminating microfilaria from the community and preventing the occurrence of new cases.

Kerala has a literate population. It also has both species of parasite, and both are vulnerable to DEC. Although the use of medicated salt does not obviate the need for mosquito control. Chertala has the special advantage that FILCO has already made mosquito control a part of people's lives by merging it with several developmental activities. Thus mosquito control becomes a by-product of the daily activities of the people.

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Searches begin and end

Bruce Ponder

THREE papers¹⁻³, to appear in the 30 September and 7 October issues of *Science*, report the identification of one gene that predisposes to breast cancer, and the first genetic mapping of another. These two genes, called *BRCA1* and *BRCA2*, together probably account for about two-thirds of familial breast cancer, or roughly 5% of all cases. *BRCA1* is also associated with predisposition to ovarian cancer.

The first evidence of linkage for a breast-cancer predisposing gene was reported in 1990 (ref. 4). The locus, on chromosome 17q, was designated *BRCA1*. Analysis of more than 200 families by an international consortium has shown that *BRCA1* is probably responsible for about one-third of families with multiple cases of breast cancer only, but more than 80% of families in which there is both breast cancer and epithelial ovarian cancer⁵. A woman who has inherited the *BRCA1* gene in an extensive family has a 60% risk of breast cancer by the age of 50, and a 90% lifetime risk; the risk of ovarian cancer seems to be similarly high in some families but much less in others, suggesting allelic heterogeneity or even two closely linked genes⁶.

Linkage mapping by the consortium rapidly refined the chromosomal location of *BRCA1* to a 1–2 megabase-pair region of chromosome 17q12–21. For the past 2–3 years, about eight groups worldwide have been engaged in a positional cloning race, which has finally been won by the Myriad Genetics Inc./University of Utah group led by Mark Skolnick¹. Their gene consists of 21 exons, distributed over more than 100 kilobases of genomic DNA adjacent to the locus *D17S855*, encoding a protein of 1,863 amino acids. On northern blots, there is a single 7.8-kilobase transcript, which is expressed in several tissues, including breast and ovary. The amino-terminal region of the protein contains a C3HC4 zinc-finger domain similar to those found in many DNA-binding proteins, whereas the rest of the protein, especially the carboxy terminus, contains an excess of acidic residues. These features suggest that the protein may be a transcription factor.

The evidence that this gene is *BRCA1* is strong. It maps to the critical region. Sequence variants found in three of eight breast or breast/ovarian cancer kindreds comprise a stop codon, a frameshift and a missense resulting in substitution of methionine by arginine. None of these variations was seen in controls, and in each family the sequence variation segregated with the disease. In a fourth kindred, polymorphisms within the coding sequence suggest that a mutation results in the absence of the messenger RNA transcript from one *BRCA1* allele. This variant also segregates with the disease. There

are too few mutations as yet to assess possible correlations with the pattern of cancers in the families.

How do the mutations affect the function of the gene? The three coding sequence mutations are consistent with loss-of-function or a dominant-negative effect. Straight-forward loss of function seems likely for the fourth mutation, in which the transcript of one *BRCA1* allele is apparently absent. This is consistent with the idea that *BRCA1* is a tumour-suppressor gene, already mooted because of the consistent finding that allele losses in the *BRCA1* region in familial tumours affect the wild-type chromosome^{7,8}, which is now open to confirmation by mutation analysis of the wild-type *BRCA1* allele in familial tumours.

At this point the story takes an unexpected twist. Futreal *et al.*³ report a search for *BRCA1* mutations in 32 apparently sporadic breast and 12 ovarian cancers, about one-third of them diagnosed in patients below 45 years of age, and all selected for having allele loss affecting one chromosome in the *BRCA1* region. If *BRCA1* is involved in the genesis of sporadic as well as familial tumours, as has usually been the case with cancer-predisposing genes, a fair number of *BRCA1* mutations would have been expected. In fact, only four were found; and, strikingly, each of these was also present in germline DNA of the same patient. This implies that *BRCA1* mutations may be relatively unimportant in sporadic cancers. One explanation might be that other genes lying in the same or alternative pathways of tumorigenesis are a more frequent target of mutation in somatic tissues. Alternatively, mutations in *BRCA1* may lead to tumour formation only if they are present at a specific stage early in breast or ovarian development. Meanwhile, the observations of frequent allele losses at or adjacent to the *BRCA1* region in sporadic tumours⁹⁻¹² suggest that there may be other tumour-suppressor genes in the region.

The second predisposing gene, *BRCA2*, has been mapped by a group based on the International Breast Cancer Linkage Consortium, and led from the Institute of Cancer Research in Sutton, UK, and from Utah². A genome-wide linkage search using microsatellite markers yielded strong evidence (LOD 11.65) for a predisposing gene lying within a 6-centimorgan (roughly 100-gene) interval on chromosome 13q12–13, centred on *D13S260*. Allele losses in sporadic tumours suggest that *BRCA2* may also be a suppressor gene; but interpretation is complicated by the closeness of another known suppressor *RBI* (ref. 13), which is, however, excluded as a *BRCA2* candidate by recombination. *BRCA2* now stands where *BRCA1* stood in 1990. If known candidate genes,

such as the putative tumour-suppressor *Brush-1* (ref. 14), are excluded, another positional cloning race is in prospect.

BRCA1 and *BRCA2* are probably of roughly equal importance in conferring risk of early-onset female breast cancer. However, no male breast cancers have been seen in *BRCA1* families¹⁵, whereas several cases are present in the *BRCA2* family set; and ovarian cancer is prominent in some *BRCA1* families⁶, but apparently much less so in *BRCA2*. At present, these differences are unexplained. Heterogeneity analysis and lack of haplotype sharing by affected individuals in some multiple-case families suggest that there are other high-risk genes.

In the short term, the practical implications of the *BRCA1* cloning are for genetic testing. Until now *BRCA1* testing using linked markers has necessarily been limited to the rather few families in which cancer could be clearly shown to be due to *BRCA1*. Even in these high-risk families, many issues are unresolved¹⁶. In particular, it is not clear what can be offered to a woman who tests positive in terms of effective screening or prevention. Experience with other adult-onset inherited disorders has shown that even a negative DNA test may not provide the expected reassurance. With the cloning of *BRCA1*, and the possibility of direct mutation testing, these uncertainties are suddenly extended to the much larger numbers of women who have only one or two affected relatives, and even perhaps to screening for *BRCA1* mutations in the population as a whole.

There are practical issues: the gene is large, and it is not yet clear how widely the mutations are scattered, and how easy or costly large-scale testing will be. It is possible that there will be a spectrum of *BRCA1* mutations associated with greater or lesser risk. Further work is needed to develop methods of early diagnosis and prevention, and to determine the best ways of informing the public and doctors. It is premature to offer population screening until these issues are resolved: but if people are to be denied access to screening, they will need to understand why. □

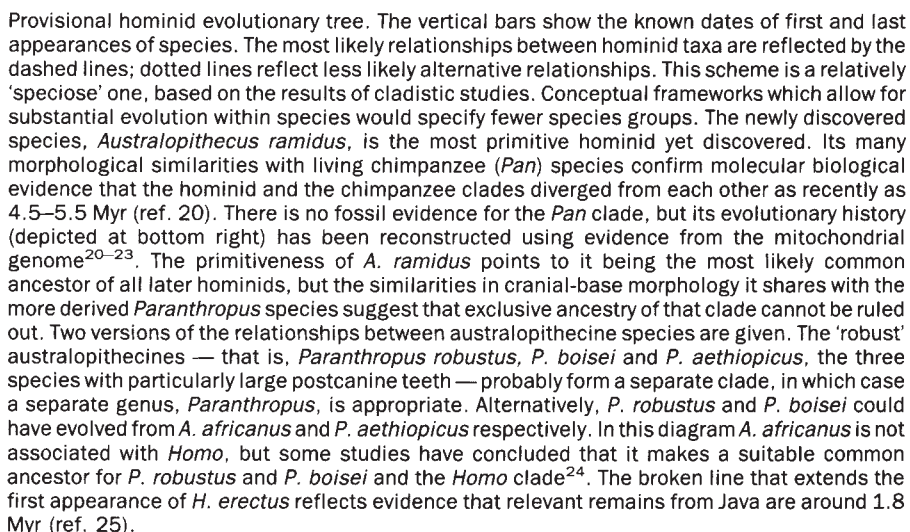
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Bernard Wood

The geological context of the fossils is described by WoldeGabriel and his colleagues². They have been able to identify a layer of volcanic ash, the VT-1/Moiti tuff, in the south-east corner of the Ara-

The preliminary palaeogeographic reconstruction of the Aramis locality sug-



gests that the hominid-bearing sediments were deposited on a flat landscape. The abundance of fossil wood and seeds, including the seeds of a genus common in forests and woodland, and the high proportion of monkey remains, which make up more than 30 per cent of the vertebrates in the faunal collection, have prompted WoldeGabriel and his colleagues² to propose that the Aramis hominids "lived and died in a woodland setting". On the face of it, their suggestion appears to be well founded. But, to be substantiated, the 'woodland hypothesis' will need to be subjected to scrutiny by researchers well versed in the biases that occur before, during and after the deposition of animal and plant remains. These taphonomic biases can so distort the fossil record that it is conceivable that the impression of woodland is the result of *post mortem* transport or the influence of predators. Even if their palaeoecological interpretation is substantiated, the authors are not the first to suggest a woodland setting for early African hominids^{7,8}, and there is increasing evidence from later in the fossil hominid record that the abandonment of some element of arboreality may have occurred as recently as around 2 Myr (see ref. 9 for review).

Of the 17 hominid specimens, all but two, VP-1/4 and VP-7/2, are cranial. Teeth predominate in the cranial remains, with the non-dental specimens restricted to a mandible fragment, VP-1/129, and the remains of a cranial base, VP-1/500. The taxonomic and functional questions arising when fossils are recovered from any fresh location are well rehearsed. Does the material belong to any existing species group? If not, can it be assigned to existing genera? What were the physical characteristics of the new species? What size was it? What was its posture, locomotion, feeding strategy and so on?

With respect to the need to recognize a new species, the taxonomic conclusions of White and colleagues are soundly based. The Aramis remains are morphologically distinct from *A. afarensis*. These distinctions are starkest for a lower first deciduous molar (dm₁) which is much more primitive in its crown morphology than the dm₁ of *A. afarensis*. Indeed, in nearly all the features that differ between *A. ramidus* and *A. afarensis*, the morphology of the former is more like that of the chimpanzee. Does this mean that it would have been more appropriate to allocate the new species to *Pan*?

In fact, the 'fit' into *Pan* is no more comfortable — indeed it is marginally less comfortable — than into *Australopithecus*. In either case homoplasies, evidence of convergent evolution, are implied. On balance the evidence of the reorganization of the cranial base with respect to that of the African apes, and the reduction in the size of the canine, together with modifica-

tions to the ulna, suggests that the hominid-like features are more likely to be derived, with the African ape-like features more likely to be either primitive retentions or examples of convergence. The evidence from the cranial base is particularly remarkable, for if the anterior border of the foramen magnum, which is the opening that transmits the spinal cord, is level with the plane that bisects the two openings that transmit the carotid arteries, then it implies a substantial shortening of the cranial base, specifically the part known as the basioccipital, comparable to that seen in *Paranthropus boisei*¹⁰. So derived a cranial-base configuration is unexpected in a hominid that is otherwise so primitive.

A more radical taxonomic solution would have been to erect a new genus for the Aramis remains; only material from other parts of the skeleton will confirm whether that would be justified. My own prejudice is that, even on the present evidence, the differences between the Aramis species and *A. afarensis* are more profound, and are more likely to reflect the sort of grade distinction that is most usefully reflected in generic distinctions, than are the differences between, say, *A. afarensis* and *A. africanus*.

The likely body size of the creatures to which the Aramis remains belong is somewhat of a puzzle, and may be another indication of the distinctive nature and adaptation of the new species. The size of the humeral head would suggest animals at least as large as the smaller individuals belonging to *A. afarensis* (that is, in excess of 30 kilograms), yet the size of the molar tooth crowns is small. Does this mean that *A. ramidus* had relatively small molar crown areas for its body mass? If so, this would distinguish it from all other australopithecines, and indeed from all other early hominids until early African *Homo erectus* or *H. ergaster*¹¹. Only in the latter species does relative molar size return to the low levels that are characteristic of living species of *Pan*. Likewise, the preliminary observations about the thickness of the enamel of the molar teeth suggests that, in this character too, the Aramis hominid shows evidence of an adaptive grade different from that of the later australopithecines.

Most significant discoveries of hominid fossils are usually, but not always justifiably, announced with the implication that they will necessitate a radical reappraisal of hypotheses of human evolutionary history. It is a sign of the growing maturity of palaeoanthropological research that, important as the discovery of *A. ramidus* is, the presence of a hominid much like it had been predicted. Since the discovery of *A. afarensis* two decades ago, opinions have been divided about its significance. Some held *A. afarensis* to be so primitive that it was treated as if it were the com-

mon hominid ancestor, or the epitome of australopithecine primitiveness^{12,13}. However, in other cladistic studies, in which no assumptions were made about its primitiveness, it was apparent that *A. afarensis* showed evidence of sufficient derived features to preclude it, in all likelihood, from being ancestral to all later hominids^{14,15}. There was, therefore, enough 'morphological space' between the hypothetical common ancestor of African apes and hominids, on the one hand, and *A. afarensis* on the other, to predict that a hominid, as yet undiscovered, would occupy it⁹.

The finds at Aramis are a vindication of proposals, based on protein morphology, that modern humans share a close and relatively recent ancestry with the African apes^{16,17}. Evidence from molecular biology has since provided powerful confirmation of that relationship, but there is no consensus about whether the closest relationship is with *Pan*¹⁸, or whether the relationship between *Pan*, *Gorilla* and the hominids is so close that its details cannot be resolved¹⁹. The wheel has come full circle — on this occasion the palaeontologists can assist the molecular biologists, for the evidence from *A. ramidus* would predict that the lineage that includes modern humans is more closely related to *Pan* than to *Gorilla*. The metaphor of a 'missing link' has often been misused, but it is a suitable epithet for the hominid from Aramis. □

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Learning from past climates

Anthony J. Broccoli

CAN information about the Earth's climate in the distant past really help us to predict the direction and magnitude of future climate change? That was the question addressed at a recent workshop* that brought together scientists from the United States and Russia, and afforded them an opportunity to compare their records of past climate. With the former Soviet Union comprising roughly one-fourth of the Northern Hemisphere land area, much of it in the climatically sensitive high latitudes, information from this vast region is important to the strategy of using palaeoclimate data to understand how the climate system responds to changes such as increases in greenhouse gases.

Information about past climates is inferred from a diverse array of biological, chemical and geological indicators such as fossil pollen grains, the shells of marine microorganisms and landforms associated with glaciation. Evidence of past radiative forcing (for example, changes in infrared-absorbing gases such as carbon dioxide, or in the reflectivity of continental surfaces) is recorded in a similar manner. Climate modellers now commonly use such information to test how well their numerical models can simulate the climate of the Last Glacial Maximum, which occurred about 20,000 years ago. Success in reproducing past climates such as this enhances the credibility of simulations of future climate change.

Sensitivity

A rather different way of estimating the magnitude of future climate change is to use reconstructions of past temperature and radiative forcing to estimate directly the global climate sensitivity, defined as the ratio of the global temperature change to the change in radiative forcing. This method, dubbed 'palaeocalibration', assumes that the relationship between global temperature and radiative forcing is nearly linear for the range of climates the Earth has experienced since the mid-Cretaceous period (about 10^8 years ago). Using information from a cold period (the last glacial) and a warm period (the mid-Cretaceous), one palaeocalibration estimate yields a climate sensitivity that would result in a warming of 2.3°C for a doubling of atmospheric carbon dioxide (M. Hoffert, New York Univ.; C. Covey, Lawrence Livermore National Laboratory)¹. This is within the sensitivity range of $1.5\text{--}4.5^\circ\text{C}$ for CO_2 doubling that has been estimated from global climate models².

A more controversial hypothesis, which has emerged from work in the former Soviet Union, is that spatial patterns of climate change may be largely independent of the nature of forcing. This 'palaeoanalogue hypothesis' is supported by climate reconstructions for a number of time periods since the mid-Cretaceous (A. Lapeis, New York Univ.). These reconstructions are unique because of the wide spatial coverage they provide and their availability for time periods much earlier than the last glacial-interglacial cycle.

The hypothesis is intriguing, as it implies that the pattern of response is an intrinsic property of the climate system and does not depend on the spatial pattern of the forcing. Furthermore, it would allow the empirical prediction of future climate change, including its spatial distribution, from predicted changes in radiative forcing due to greenhouse gases without the use of climate models^{3,4}. As described at the meeting, the 'universal temperature response' implied by the palaeoanalogue hypothesis has been compared with climate changes simulated by models, and potentially significant differences are indicated. The universal temperature response tends to be larger than that of climate models in polar regions and smaller than the model response at low latitudes (K. Selyakov, State Hydrological Institute, St Petersburg).

But we shouldn't throw out our climate models yet. Some are sceptical of the climate reconstructions that have been used to develop the palaeoanalogue hypothesis, for a number of reasons. First, the temperature estimates are typically presented as contoured maps or averages around the latitude circles. Do enough data really exist to reconstruct past global temperature distributions, particularly for distant time periods such as the Cretaceous and early Tertiary? Second, as raw palaeoclimate data consist of proxies that are assumed to respond to climate, a concern is whether the procedures for deriving temperature estimates from these proxies are objective and well documented. Finally, both the basic data (such as pollen counts in lake sediments) and stratigraphic information required to establish their chronology have not been readily available to palaeoclimate specialists outside the former Soviet Union.

The verdict on the palaeoanalogue hypothesis must await the resolution of these issues, as it depends heavily on the reconstructions developed in the former Soviet Union. This cuts both ways: the utility of palaeocalibration also depends on the same reconstructions, because they

are a potential source of information about additional episodes in the Earth's climate history. The more information we have, the better we can quantify the relationship between global radiative forcing and temperature response. Climate modellers would also benefit from the availability of quantitative climate reconstructions from times when the Earth was warmer than today. These would supplement the better-documented variations that have occurred during the last glacial-interglacial cycle.

Integration

For full value to be obtained from the palaeoclimate information developed in the former Soviet Union, the data will need to be converted into computer-accessible forms and integrated with existing worldwide archives; and methods for deriving climate variables such as temperature and precipitation must be compared, to resolve possible conflicts in interpretation. Support for such research is in short supply, given the economic difficulties faced by present-day Russia, and workshop participants asked that their expression of support be directed to those United States government agencies funding climate research.

If successful, will the palaeoclimate data produced by such an effort help us to quantify global climate sensitivity, and what are the alternatives? Perhaps the best way to calibrate sensitivity would be through the contemporaneous simulation and monitoring of ongoing changes in climate, but because of inadequacies in measurements of past variations in both climate forcing and response, improved systems for climate monitoring would be required before we could adopt this strategy⁵. Even if such systems existed today, several more decades might be required to collect enough data. In the meantime, we may need to look to the prehistoric past to assess the quality of current estimates of climate sensitivity. Although the uncertainties in attempting to deduce variations in climate during such distant times probably preclude fine-tuning these estimates, careful examination of palaeoclimate data should increase either our confidence or our scepticism. □

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* Workshop on Palaeocalibration of Climate Sensitivity, Silver Spring, Maryland, USA, 15–17 August 1994.

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Superantigen as suspect

H. Robson MacDonald and Hans Acha-Orbea

INSULIN-dependent diabetes mellitus (IDDM) is a T-cell-mediated, autoimmune disease of unknown origin that results in destruction of pancreatic islet β -cells in genetically predisposed individuals. On page 351 of this issue¹, Conrad *et al.* provide provocative evidence that expression of a superantigen by pancreatic islet cells may be an important factor in IDDM aetiology.

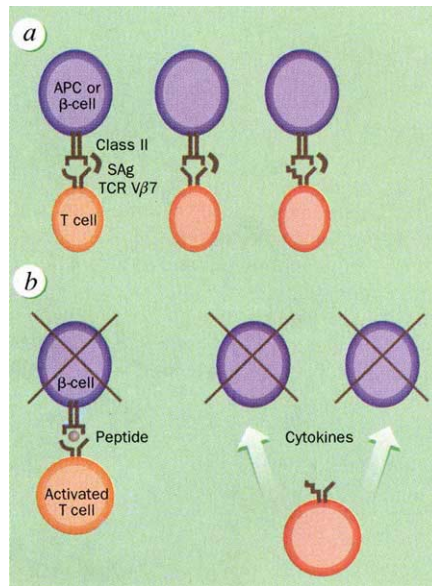
The general idea that superantigens may be involved in autoimmune disorders has been around for some time. The foundation for such speculation is based primarily upon a unique property of superantigens — that is, their ability to activate a large proportion (up to 20 per cent) of T cells in a given individual through interaction with the variable domain of the β -chain (V β) of their antigen receptor². In contrast, conventional foreign antigens (and presumably by analogy autoantigens) only stimulate a minute fraction of T cells (estimated to be 1×10^{-4} – 1×10^{-6} in an unprimed individual) because they interact with a much more restricted T-cell-receptor structure determined by V β and V α domains in addition to other hypervariable elements.

How could this polyclonal T-cell-activating property of superantigens be responsible for the onset of a tissue-specific autoimmune disease such as IDDM? There are at least two possible explanations (see figure).

In the first model, one could postulate the existence of a small number of T cells specific for self-antigens expressed by pancreatic β -cells. Normally these cells would be inoffensive because autoreactive T cells are functionally inactivated (or eliminated) by a properly functioning immune system. If, however, pre-diabetic patients were exposed to a superantigen, those T cells bearing superantigen-responsive V β domains would become activated. Obviously this large population of activated cells could potentially include the rare T-cell subset with antigen receptors specific for pancreatic self-antigens, if the latter cells happen to express the same V β domain. In this way, T cells activated by a superantigen could mediate specific tissue damage. This model resembles the so-called 'molecular mimicry' theory of autoimmunity³ in which exposure to an infectious agent triggers T cells with antigen receptors that recognize shared epitopes expressed by autoantigens and microbial proteins; however, in the case of superantigens, no such molecular mimicry would be required to initiate autoimmunity.

An alternative model would be to invoke nonspecific mediators of tissue dam-

age such as cytokines. Indeed, pancreatic islet β -cells appear to be unusually sensitive to the cytotoxic effects of cytokines such as interleukin-1, interferon- γ and tumour necrosis factor- α , at least as assessed *in vitro*^{4,5}. These (and other) cytokines would be expected to be produced upon superantigen stimulation *in vivo*,



Models for the involvement of superantigen (Sag) in IDDM. *a*, T cells expressing the superantigen-reactive T-cell receptor (TCR) V β 7 are stimulated by antigen-presenting cells (APC) or pancreatic β -cells expressing the superantigen and major histocompatibility complex (MHC) class II. Superantigens bind to MHC class II molecules and are able to interact with both CD4⁺ (helper) and CD8⁺ (cytotoxic) T cells expressing these V β s, leading to their activation and increase in size and number. Activation by APC can take place either in the pancreas or in the periphery. *b*, Destruction of pancreatic β -cells can occur by MHC-restricted, cell-contact-mediated recognition of β -cell-specific peptides by rare superantigen-reactive T cells which cross-react with pancreatic autoantigens; or it can occur by release of large quantities of cytokines by superantigen-activated T cells. MHC class II expression by pancreatic β -cells *in vivo* is still controversial.

potentially leading to selective damage of bystander islet cells.

Although the arguments advanced by Conrad *et al.*¹ in favour of a general superantigen involvement in IDDM are thought-provoking, they should be hedged with caveats. First, the authors have data on only two, rather unusual, IDDM patients who died rapidly after apparent onset of the disease. Second, neither the superantigen itself nor its putative causative agent has been identified. In the absence of more direct evi-

dence, their conclusion thus rests on the interpretation of observations that T cells bearing a particular V β domain (V β 7) selectively infiltrate diseased pancreatic islets. In view of the numerous examples of V β -restricted T-cell responses to conventional antigens and autoantigens⁶, it could be formally argued that the islet-infiltrating V β 7⁺ cells seen by Conrad *et al.* are in fact specific for a β -cell autoantigen. This interpretation seems unlikely, however, because antigen-specific V β -restricted responses of this magnitude would be expected only following specific *in vivo* priming, whereas Conrad *et al.* observe that V β 7⁺ cells from non-diabetic individuals expand dramatically when cultured with diseased pancreatic membranes. Furthermore, the extreme diversity of V β 7 junctional sequences observed by Conrad *et al.* is most easily reconciled with a superantigen effect (although similar sequence heterogeneity has been seen for at least one V β -restricted antigen-specific response⁷).

It is instructive to consider rodent models of T-cell-mediated autoimmune disease where the effect of superantigens can be directly tested. Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system. In certain genetically susceptible strains of mice and rats, EAE is a chronic disorder that is mediated primarily by T cells that recognize myelin basic protein via T-cell receptors that are biased towards V β 8.2 (ref. 6). Importantly, clinical symptoms of EAE can be re-induced in cured mice by injecting a superantigen (staphylococcal enterotoxin B) that specifically activates V β 8.2⁺ T cells^{8,9}. Unfortunately, no such experiment has been carried out in the non-obese diabetic (NOD) mouse, a rodent model for IDDM, where the V β repertoire among T cells infiltrating diseased pancreatic islets appears to be unbiased^{10,11}.

If indeed pancreatic islets of IDDM patients express a superantigen, it will be of considerable theoretical and practical importance to characterize it biochemically. All superantigens identified to date are

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the products of either bacteria or viruses², raising the obvious possibility that the IDDM-associated superantigen is of infectious origin. In this regard there is evidence for upregulation of expression of endogenous retroviruses in diseased pancreatic β -cells in certain mouse models¹², and infection with some viruses (such as congenital rubella) increases the risk of IDDM in genetically susceptible individuals¹³. As Conrad *et al.* point out, the putative infectious agent encoding the

superantigen could either reside in pancreatic β -cells or alternatively be transported to the islets by infected lymphocytes. Whatever the explanation, the race will soon be on to identify this islet superantigen and to determine more precisely its role in the aetiology of IDDM. $\square$

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MOLECULAR SELF-ASSEMBLY

Snap-together vesicles

Helmut Ringsdorf and Joachim Simon

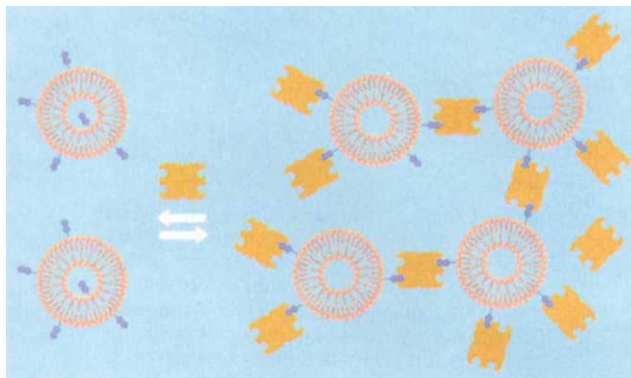
IN cell membranes, mother nature demonstrates the perfect interplay of molecular recognition and molecular self-organization to assemble systems whose function is based on their organization. Science has lagged far behind. Until recently, attempts to use the principles of self-organization, regulation, replication, communication and cooperativity to build up structures have remained at the level of systems such as monolayers, vesicles or micellar aggregates. Interest is growing in molecular engineering of the next level of supramolecular systems — where already complex self-organized aggregates are combined to yield larger functional assemblies. The aim is to mimic the fascinating pathways of nature where molecules, macromolecules and molecular devices can be seen as merely the building blocks for cells, cell complexes, organs and organisms.

Chiruvolu *et al.*¹ have now combined two well-known and well characterized model systems, namely vesicles and biotin-streptavidin as recognition-units. They have looked into a neglected application for these systems: the formation of cross-linked vesicular aggregates by site-specific recognition.

First, they prepared small unilamellar vesicles from mixtures of naturally occurring phospholipids and biotin-functionalized lipids. Biotin (vitamin H) binds to a tetravalent protein (Streptavidin) from the bacterium *Streptomyces avidinii* with a binding constant of $k_a = 10^{15} \text{ M}^{-1}$. The popularity of this system originates from its widespread usage in affinity chromatography². Upon addition of the tetravalent protein to the vesicular dispersion, the vesicles aggregate and precipitate, as shown schematically in the figure.

This recognition experiment is thus

far merely a variation on a theme that has been extensively investigated in liposomes³ and monolayers^{4,5}. Only the lipid structure and vesicle composition were slightly changed. But it became very interesting upon closer investigation. Cryo-microscopical examination of the aggregates revealed tethered liposomes



Schematic illustration of vesicle aggregation induced by specific recognition of biotin lipids by the tetravalent protein streptavidin.

linked to each other by biotin-streptavidin bridges, but which have retained their spherical shapes and seemed to have mostly unperturbed membrane surfaces.

In contrast, liposomes that have been aggregated under osmotic stress or by addition of calcium ions^{6,7} have to undergo dramatic deformations to maximize the attractive forces between single vesicular compartments. Such deformations make the vesicle membranes fragile and leaky, and sometimes lead to fusion of membranes, reducing the prospect of using assemblies of vesicles, for example as

encapsulation composites.

With the new, site-specific binding between vesicles, the build-up of secondary supramolecular structures looks to be within reach, especially as the composition and thereby the distribution of binding sites in vesicular systems is easily controllable. And the aggregation is reversible: on competitive replacement of the lipid-bound biotin, the liposome aggregates could be redispersed without losing the integrity of the vesicular compartments. One of us (H.R.) found similar results several years ago using coloured vesicles prepared from polydiacetylene lipids with sugar headgroups. The coloration allowed us to follow the reversible aggregation of the intact vesicles by specific binding of lectins such as Concanavalin A⁸. Chiruvolu has calculated the binding forces between the tethered liposomes, and found that one specific binding site between two liposomes is energetically comparable to the strongest binding interactions attainable with nonspecific colloidal forces⁹ — but without the disadvantages that nonspecific aggregations bring about.

Specific, reversible cross-linking of vesicular assemblies looks promising as part of experiments designed to examine specific binding events and binding forces¹⁰, as the size of the vesicles, their degree of functionalization or the inclusion of probes can be easily controlled¹¹. Even more exciting would be the possibility of constructing soft biomaterials with tunable properties. Cross-polymerization of the lipids, and the use of ligand-functionalized hydrophilic polymers as cross-linking matrices, can strengthen and stabilize the assembly but allow us to retain control of the bulk properties by manipulating osmotic forces — swelling or shrinking will influence the elastic properties of the material. Innumerable possibilities exist if we can combine liposomes or polymerized liposomes with polymeric materials. It will be exciting to follow whether these concepts will finally lead to artificial tissue-like composites. $\square$

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Linus Pauling (1901–1994)

LINUS Pauling died on 19 August at his home in California at the age of 93. He was widely regarded as the greatest chemist of the twentieth century — such was the depth of his intuitive understanding of the subject that his co-workers felt he sometimes knew the answer to a question before developing a theory to explain it.

Pauling's scientific work had a special style. There was frequently a boldness in his proposal of new ideas, often at first thought outrageous for the simplicity with which they explained complicated phenomena. The essence of his approach to chemistry lay in the way in which he related quantitative physical chemical measurements, especially molecular structure parameters, to theoretical interpretations. Pauling was aided by his enormous knowledge of chemical properties, his phenomenal memory, and a theoretical understanding that allowed him to make often unexpected correlations between physical measurements and chemical properties. An example is his early work in the late 1920s in formulating a simple set of rules governing the structure of ionic minerals, especially silicates.

Likewise, in his work on the nature of the chemical bond, Pauling started with such facts as bond distances, bond angles and molecular dipole moments — the elementary data of structural chemistry — and, applying quantum mechanics, developed a theoretical structure based on the hybridization of orbitals that predicted the directed valence of atoms. This work was an aid in predicting the chemical properties of molecules and also established an outlook that has influenced nearly everyone who subsequently entered this field of science. This is simply the attitude that one can explain and correlate by theory the many diverse physical and chemical properties of atoms and molecules.

In 1939 Pauling published *The Nature of the Chemical Bond*. More than any other, this book revolutionized the study of chemistry. It presented a way of understanding chemistry based on rational physical principles and drove home the importance of thinking in three dimensions. The 1954 Nobel Prize for Chemistry was awarded to him largely in recognition of these contributions.

In the 1930s Pauling's interest began to spread beyond small molecules to haemoglobin and antibodies. The nature of proteins was not understood at that time. He recognized the central role of hydrogen bonding and other weak interactions in determining and maintaining macromolecular structure. In 1940 he and Max Delbrück developed the idea of the importance of molecular complementarity in macromolecular interactions. They sug-

gested that such interactions were, very probably, an essential part of the molecular basis of genetics.

Perhaps one of the best examples of Pauling's audacious thinking was in his proposals enumerating the possible structures of polypeptides, including the α -helix and pleated sheet. These discoveries were made through intuition and model building — all this being done, as Pauling said, "with one's feet higher than one's head, for convenience" (he often did his best think-

IMAGE
UNAVAILABLE
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REASONS

Pauling — "a real genius".

ing with his long legs propped up on his desk). The rules for determining possible configurations were simple, and, in essence, they minimized the total energy of the structures. His approach to structure solving has served as a model for many workers in the field, including those who have solved the structure of a variety of polymeric molecules ranging from DNA and collagen to polypropylene.

In 1945, Pauling attended a lecture in which William Castle described sickle-cell anaemia, a disease characterized by intracellular aggregation of haemoglobin molecules in non-aerated blood. While listening to the lecture, he imagined that this phenomenon might be explained by a hereditary alteration of the surface of a haemoglobin molecule that would make it complementary to another part of the surface, so that in the absence of oxygen these altered haemoglobin molecules might combine together. This idea was the beginning of our understanding of molecular disease.

A frequent characteristic of Pauling's work was that, after his interpretation of a phenomenon, its acceptance became widespread and it began to appear obvious. One example was his description, in 1946, of the importance of the surface of proteins in determining specificity through complementarity of shape. He also realized that enzyme action could go in both

directions, and thus it seemed apparent to him that what the enzyme had to do was lower the activation energy for the transition. Another example was his work with Zuckerkandl in 1962. By looking at changes in the amino-acid sequence of haemoglobin in different animals, they could correlate the changes with the evolutionary period at which the animals diverged from each other. This suggested that mutations accumulate in a fairly regular manner with time in a particular protein and therefore that they could be used to measure evolutionary time. This principle was the beginning of the field of molecular evolution. It was a startling idea when it was presented. It is now so widely accepted that it seems almost self-evident.

In 1948, Pauling described the gene as two complementary units held together by weak forces such as hydrogen bonding. Although he failed to solve the structure of DNA, Watson and Crick's achievement in doing so owes much to Pauling's methodology.

Pauling was a warm, compassionate man, who cared deeply for people who were oppressed for their political beliefs. With the development of huge nuclear arsenals after the Second World War, he became an active campaigner for peace, stimulated by his wife Ava Helen. Here, too, his intuition played a key role. He became convinced that radiation was harmful to the genome, even in small amounts, and crusaded tirelessly for a cessation of nuclear testing. Today, it is generally understood that radiation from radioactive fallout or X-rays is harmful. But when Pauling started his campaign, the idea was regarded as outrageous, and even subversive. In 1962 Pauling won the Nobel Peace Prize for his efforts in helping to bring about a Test Ban Treaty.

By the 1970s Pauling's attention turned to antioxidants — vitamin E and especially vitamin C. His analysis and his intuition led him to the conclusion that we would be healthier if we took supplements of these vitamins. The debate on the role of antioxidants in general and vitamin C in particular continues; however, many analyses are emerging which support Pauling's views on the importance of these compounds.

Linus Pauling was widely honoured. In addition to two Nobel Prizes he received over 50 medals and awards from a great variety of organizations, and almost as many honorary degrees from universities. The esteem with which he was regarded was vividly illustrated to me in 1951 when, as a postdoctoral fellow of Pauling's, I visited Albert Einstein in Princeton. Einstein's comment to me was "Ah, that man is a real genius!" Alexander Rich

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Roger Reesmyer Starlight/SPL

cAMPing out

Leland Hartwell

CELLS often exhibit a remarkable ability to recover from insults, hinting at the existence of homeostatic mechanisms that return them to an equilibrium state. This point was powerfully demonstrated¹ almost 40 years ago. When a portion of the cytoplasm of a growing amoeba was repeatedly cut from the cell, the cell continued accumulating mass, but never divided — apparently it was aware that it had not reached a size sufficient for division.

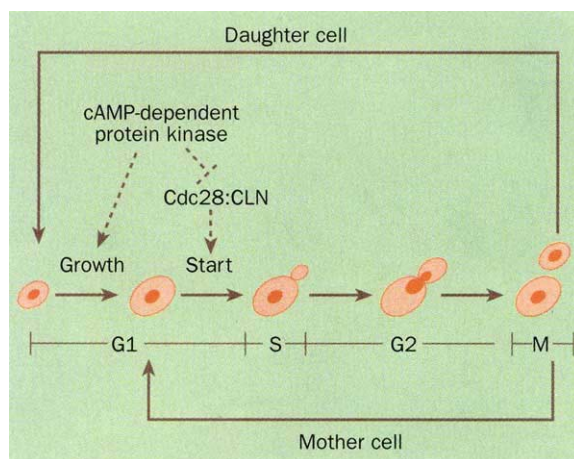
Two reports on pages 339 and 342 of this issue^{2,3} uncover a new role for cyclic AMP in the homeostatic mechanism that integrates cell growth with division. They deal with the budding yeast, *Saccharomyces cerevisiae*, which has been a popular organism for studies on the relationship between growth and division in part because the new growth in each cell cycle is visible as a distinct body, the bud, that becomes the daughter cell.

A mother cell begins the cell cycle in G1 phase; it produces a new bud concomitant with S phase; the bud grows throughout S and G2; the nucleus divides at M with one nucleus going to the mother and one to the bud, and the cell then divides (see figure). Growth is integrated with division in G1. This is most evident from the different behaviours of the mother cell and daughter bud in the next cell cycle. The daughter is normally smaller than the mother cell at division, especially under conditions of slow growth. The daughter cell delays more than the mother in the G1 phase of the next cell cycle before producing a bud, giving itself time to grow to the size characteristic of a mother cell⁴. There is no requirement for growth in other phases of the cell cycle because, if growing cells are suddenly starved of nitrogen to prevent protein accumulation, all cells that have budded complete the cell cycle and divide without net growth⁵. Buds that were small at the time of starvation produce tiny cells which, upon readdition of nitrogen, stay in G1 until they have reached the characteristic size of mother cells.

These observations suggest that cells must simply accumulate enough stable protein in order to pass from G1 to S. Others, however, indicate that the control is more dynamic, responding to the rate as well as the amount of growth. If the rate of protein synthesis is limited in specific ways, for example, then cells can greatly exceed the critical size that normally char-

acterizes the G1/S transition⁶. Moreover, cells have characteristically different sizes with different carbon sources and, when G1 cells are shifted from one carbon source to another, they adjust to the new size requirement for budding before moving on to S phase⁷. From these findings it would seem that the critical component monitoring growth is unstable.

Molecular definition of the homeostatic mechanism that integrates growth with



The cell cycle consists of G1, S, G2 and M phases. In *Saccharomyces cerevisiae*, growth is coordinated with division in the G1 interval. Small daughter cells, produced under conditions of growth limitation, do not pass Start until they have achieved the size of mother cells. cAMP-dependent kinases have antagonistic roles at Start, being required for growth and inhibiting the transcription of cyclins.

division will require knowledge of the molecular components that mediate 'growth' and 'division', respectively, and those that communicate between them. Previous work implicated the cAMP-dependent protein kinases as important mediators of growth, and the cyclin-dependent kinases as mediators of division. The two new papers^{2,3} provide a link between them. A variety of mutations that inactivate either the production of cAMP or their targets, the cAMP-dependent protein kinases, result in a cessation of growth and arrest of the cell cycle in G1 (ref. 8). Mutations that inactivate the *S. cerevisiae* cyclin-dependent kinase Cdc28 arrest the division cycle in G1 while growth continues⁹. The G1 collaborators of the cyclin-dependent kinases, the cyclins CLN1, CLN2 and CLN3, are metabolically unstable, being synthesized in G1 and degraded at the G1/S boundary. The cyclins are therefore prime candidates for the dynamic component monitoring size control; indeed, cyclin deficiency can produce larger than normal cells¹⁰ and cyclin excess smaller than normal cells¹¹.

Baroni *et al.*² and Tokiwa *et al.*³ use

different approaches to come to the same conclusion — that cAMP-dependent protein kinases inhibit transcription of the *CLN1* and *CLN2* genes. Moreover, although transcription of *CLN3* is not subject to this inhibition, it may play a special role in mediating the integration of division and growth through the products of *CLN1* and *CLN2*. The addition of cAMP to the culture medium of cells under special physiological conditions or for cells altered in cAMP uptake or metabolism results in G1 arrest, growth that exceeds the normal size at budding, and inhibition of *CLN1* and *CLN2* transcription. Expression of *CLN1* or *CLN2* in these cells under the control of a promoter not subject to cAMP regulation overcomes the G1 arrest, showing that G1 arrest results from failure of *CLN1* or *CLN2* protein to accumulate. Mutations in the regulatory subunit of the cAMP-dependent kinases or in the kinases themselves demonstrate that these effects are being mediated through the cAMP-dependent kinases. Hence these experiments show that cAMP exercises negative control on division through the cyclins, whereas previous experiments, mentioned above, demonstrated that it has positive control on both growth and division.

How growth and division are coordinated through these antagonistic effects remains to be seen. One clue comes from work showing that reducing the rate of initiation of polypeptide chains can arrest cells in G1, in spite of the fact that the cells grow larger than the unperturbed G1 cell, whereas limiting the rate of polypeptide elongation does not have this effect⁶. So one important task will be to define the relationship between the initiation of protein synthesis, cAMP metabolism and expression of *CLN* genes. □

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The common stuff of stardust

George J. Flynn

INTERSTELLAR silicates may have been identified in interplanetary dust particles collected by aircraft in the Earth's stratosphere, according to a report by John Bradley in *Science*¹. Although exotic interstellar grains have previously been found in meteorites, the amorphous silicates that he has identified could well be the common type of interstellar dust, observed, for example, in protostellar and protoplanetary nebulae, and about supernovae. The silicates were identified in primitive anhydrous interplanetary dust particles (IDPs). These IDPs are aggregates whose basic subunits are rounded, amorphous silicates less than a micrometre across and containing metal and sulphide crystals^{1,2}. Bradley names them 'GEMS', an acronym for glass with embedded metal and sulphides.

Characterization

The identification of GEMS as interstellar dust is based on chemical evidence indicating that they were strongly irradiated by ions before their accretion, as well as on their similarity to the properties of interstellar dust inferred from astronomical measurements. Bradley used energy-dispersive X-ray analysis to obtain profiles of element abundances in the GEMS which showed depletions in magnesium and silicon near their surfaces. He found similar element depletions in lunar soil grains irradiated by the solar wind and in a laboratory-irradiated sample of the Allende meteorite.

In both the latter cases, irradiation by ions causes an initially crystalline silicate to become amorphous, produces metal and sulphide crystals distributed throughout, and strips those cations with the weakest bond strengths (first Mg and Ca, and later Si) from the material. Thus, Bradley concludes the GEMS were heavily irradiated; and as these depleted particles are distributed throughout the IDPs the irradiation must have occurred before accretion.

Preaccretionally irradiated grains have been found before now in meteorites. These grains are scarred by the tracks left by large numbers of energetic particles from solar flares and contain high abundances of solar-wind-implanted noble gases³. What seems to have happened is that these meteorite grains were exposed to the solar wind in the parent body regolith, or loose dusty coating, before incorporation into the meteorites. Irradiation in a regolith is also possible for the GEMS. However, Bradley observes that the GEMS are more severely chemically altered than the surfaces of IDPs, which were exposed to the solar wind for about

10^4 years between release by their parent bodies and their collection on Earth. So the GEMS would appear to have been exposed for far longer times, much more than 10^4 years in the current solar flux. Solar-wind ions penetrate less than a micrometre into solid material, so the GEMS would have had to reside directly on the surface of the parent body for this time. But the regolith of objects in the inner Solar System is continuously stirred to depths from millimetres to centimetres by impacts of micrometeorites and the surface material is buried by the ejecta from the less frequent impacts of larger meteorites. This limits the surface exposure of most particles in the regolith. Modelling of asteroidal regoliths and measurements on lunar soil samples suggest that, with the current ratio of solar wind to micrometeorites, most grains would reside on the surface for too short a time to produce the chemical effects measured by Bradley.

Interstellar grains have previously been extracted from meteorites. Diamonds less than 5 μm in size were the first interstellar grains identified by the unusual composition of their noble gases⁴; identifications of interstellar silicon carbide, graphite and alumina have followed⁵. But these are exotic phases, not the common dust in the interstellar medium.

What do we already know about interstellar dust? Infrared spectra of dust in protostellar objects and molecular clouds both show a silicate feature at a wavelength of 10 μm , consistent with laboratory spectra of silicate glasses⁶. The chemistry of interstellar grains, derived from element depletions in interstellar gases, is inferred to be roughly chondritic^{7,8}. The size distribution of interstellar grains⁹, derived from stellar extinction, peaks at about 0.1–0.2 μm . If these radiation-damaged, submicrometre, silicate glasses were incorporated into meteorites they would, most probably, have been altered by the subsequent aqueous or thermal processing experienced by even the most primitive meteorite matrices.

Brownlee has frequently suggested that the most likely place to find surviving interstellar silicates is in the least altered extraterrestrial material available for laboratory analysis — the primitive, anhydrous IDPs¹⁰. The non-equilibrium chemistry and mineralogy of these IDPs indicates that they have experienced less thermal and/or aqueous alteration than meteorites or hydrated IDPs. GEMS appear to be unique to the primitive IDPs, and make up the bulk of the mass of many anhydrous IDPs. As Bradley now shows¹,

these GEMS are roughly chondritic (except for carbon) in major element contents, and range from 0.1 to 0.5 μm in size. Thus, the properties of the GEMS are consistent with those inferred for interstellar silicates, and their presence in only the least altered extraterrestrial material is consistent with the expected ease of aqueous or thermal alteration. If Bradley's identification of GEMS is correct, then some anhydrous IDPs are primarily aggregates of surviving interstellar dust.

The isotope anomalies that allow one to identify the interstellar grains from meteorites provide no definitive answer for the GEMS. Isotopic measurements¹¹ on individual IDPs (it is not yet possible to look at individual GEMS) show excesses of D and ¹⁵N, but no significant deviations from the terrestrial isotopic ratios of C, O or Si. Similar D and ¹⁵N excesses are observed in primitive matrix material of meteorites, causing Walker¹¹ to suggest that chondritic IDPs are no more primitive, at least in the isotopic sense, than meteorite matrices — and these are believed to be nebular, not interstellar, condensates. But the absence of isotopic anomalies does not prove that the IDPs are dominated by Solar System condensates. The Solar System itself was formed from interstellar gas and dust, so the average isotopic composition of the interstellar material in the region of formation was 'solar'.

Alternatives

Now for a couple of caveats. First, the long radiation exposure time inferred for the GEMS assumes the present solar radiation flux. Much shorter exposures would be required during an era of more intense flux earlier in the Sun's history. The GEMS could have condensed from the solar nebula, been irradiated during the Sun's T-Tauri phase, and accreted to form IDPs. If so, then the GEMS would be surviving solar nebula condensates, rather than interstellar grains. Detailed modelling of the behaviour of dust and the intensity of radiation during the T-Tauri phase will be needed to rule this out.

Second, Keller *et al.*¹² previously proposed another origin for the globules that Bradley calls GEMS, as agglutinates pro-

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duced by micrometeorite impacts into the regolith of the parent body. Lunar agglutinate glass contains metallic iron and sulphides, like the GEMS. Keller and McKay also observed compositional differences between the rims and centres of lunar crystals¹³ which they attributed to deposition of vapour from nearby impacts. If both the mineralogy and chemical gradients of the GEMS can be produced by impact-derived regolith processing, then a preaccretional irradiation may not be required. However, Bradley indicates this mechanism will produce oxygen depletions, rather than the excesses (or Mg and Si depletions) he finds¹.

If it can be confirmed that the only

plausible mechanism to produce these chemical gradients is exposure to intense radiation, then it seems these amorphous glasses are either the long-sought interstellar silicates or the earliest known nebular condensates. In either case, detailed study of these unusual IDP building blocks should improve our picture of the process of Solar System formation, and provide laboratory samples against which to compare the emission features observed in astronomical settings. □

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GEOPHYSICS

A mystery in the mantle

John E. Vidale

THE rocky mantle occupies more than 80 per cent of the volume of the solid Earth, and excludes only the 10–100-km-thick crust on the surface and the 3,000-km-radius iron core at the centre. Although the mantle is known to flow with the convective currents that release the Earth's heat to space, the simplicity of the flow pattern is a subject of debate. The question of flow in the mantle hinges on the difference between the upper and lower mantles. Some geochemical evidence suggests differing compositions for the two mantle layers, implying separate convection cells in each, whereas other evidence from seismic tomography suggests whole-mantle convection, implying a homogeneous composition. To muddy the waters still further, Kawakatsu and Niu, on page 301 of this issue¹, now throw in an observation of mantle layering at far greater depths than had been expected.

The search for the possible compositional boundary between the upper and lower mantles has tended to concentrate near 660 km depth. But we now attribute the observed changes in seismic velocities and density there to a phase transformation^{2–8}, despite some lingering inconsistencies^{9–11}. The signature of the proposed compositional contrasts should be more subtle, and might coincide with the phase transition or might be deeper. The geophysical signs of upwellings have proved tantalizingly faint, so studies have focused on the more visible downwelling subducting slabs.

Recently, strong evidence has been presented for simple mantle downwelling beneath the west coast of the Americas that extends to the base of the mantle^{12,13}. More complicated geometries have been inferred from seismic observations in the western Pacific as well as from some flow

models^{14–16}. It has been proposed that the phase transition near 660 km depth may hold back downwellings for millions of years, followed by episodes with much material flowing into the lower mantle. This theory of alternating accumulation and flushing of downwellings has been termed dynamic layering, but like simple whole-mantle convection would probably homogenize the compositions of the upper and lower mantles.

Kawakatsu and Niu¹ find layering beneath several subduction zones that is deeper, about 920 km down. As they discuss, structure at this depth has been previously proposed¹⁷, but the evidence is now far more compelling. The J-array, the network of short-period seismometers that they used, is much longer than those of previous high-frequency studies of this type^{4,6,8}. The 4,000-km aperture allows unprecedented resolution in unscrambling high-frequency reverberations, and the ray paths from Tonga to Japan sample parts of the mantle unreached by previous studies.

The origin and extent, and thus the implications, of the layering seen near 920 km depth are not yet clear. It may be the signal of a phase change related to the increase in pressure with depth in the mantle, such as a change in structure of perovskite or reactions involving garnet in the downwelling slab. The former would be present globally, whereas the latter would appear only near slabs. However, these effects were not necessarily expected to appear at this depth, nor would they be expected to produce a signal as large as observed.

Kawakatsu and Niu favour the suggestion that the layering is the signature of a global change in composition with depth. But this too has its difficulties. A chemical boundary would be expected to fluctuate

in depth by hundreds of kilometres, even for the larger density contrasts present at the 660-km boundary^{5,7}, in response to the convective currents. Instead, the observations suggest a fairly constant depth for the boundary.

In addition, a global compositional boundary would coincide with a thermal boundary layer, probably with a temperature contrast of several hundred kelvin, because the transfer of heat by convection would be interrupted by the compositional change. The large gradient in temperature near a thermal boundary layer results from the relative inefficiency of conductive heat transfer. The extra temperature increase with depth would be consistent with high estimates of core temperatures¹⁸, but should also enhance the visibility of the structure to seismic waves, whereas the 920-km-depth layering is subtle. Other iron studies indicate lower core temperatures, compatible with whole-mantle convection¹⁹.

Another problem is that mantle downwellings that pass through this depth and would cause mixing between the layers have been observed^{12,14}. And the presence of a global boundary with a several per cent contrast in velocity across a 10-km depth interval, as the authors suggest, would generate complications in grazing P waves that are not generally observed.

So the cause of Kawakatsu and Niu's structure at 920 km depth is not yet certain. What is clear is that the more carefully we look, the more structure becomes visible within the Earth. And as usual, it appears that seismic observations are ahead of the theories. □

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Core correlations

Rainer Zahn

THE past two years have seen a revolution in our thinking on climate variability, largely because of the signs of ultra-fast climate oscillations found in deep ice cores. But the ice-core records for part of the last interglacial period disagree, and other evidence for the variability of the climate during this period has been eagerly sought — after all, it might be a model for our own interglacial. On pages 323 and 326 of this issue, Keigwin *et al.*¹ and McManus *et al.*² provide welcome evidence for how far oceanic records from the same period correlate with the ice-

core data.

The story begins in September 1992, when the first results to emerge from the Greenland Ice-Core Project (GRIP), one of two international teams drilling at Summit in Greenland, indicated that the last glacial period had been punctuated by surprisingly warm intervals³. Within months, results from the nearby GISP2 core⁴ had confirmed this and shown that ultra-fast, high-amplitude climate 'flickers' were common in the glacial period⁵. So a vigorous search started among marine palaeoclimatologists and mod-

ellers for clues to how the oceans' circulation might have contributed to these climatic lapses.

In September last year, Bond *et al.*⁶ obtained high-resolution records of planktonic foraminifera abundances from a North Atlantic sediment core, and found that these were a near-perfect match with the ice-core records over the past 90,000 years (back through the last glacial period, but not the previous interglacial). The foraminifera abundances vary according to ocean surface temperature, so this provided part of the urgently sought link with the North Atlantic's thermohaline circulation. Bond *et al.* suggested that glacial climates were repeatedly perturbed by collapses of the North American ice sheet: huge iceberg surges flooded

Linking ice-core records to ocean circulation

Shown here are the positions of the Greenland 'Summit' ice cores (labelled 1) and the sediment cores used by Keigwin *et al.*¹ (4) and McManus *et al.*² (2 and 3) on pages 323 and 326 of this issue to monitor the North Atlantic's circulation during the last interglacial. The North Atlantic polar front (the circulation boundary between cold polar and warm boreal water masses) may be viewed as a door which opens and shuts in the course of climate shifts between interglacial and glacial conditions.

For our present age, the Holocene (left), the ice-core record implies a stable interglacial mode with no major perturbations (BP, before present). The 'door' in the North Atlantic is wide open and allows warm subtropical water masses to flow far to the north, thus driving the thermohaline circulation and heating up

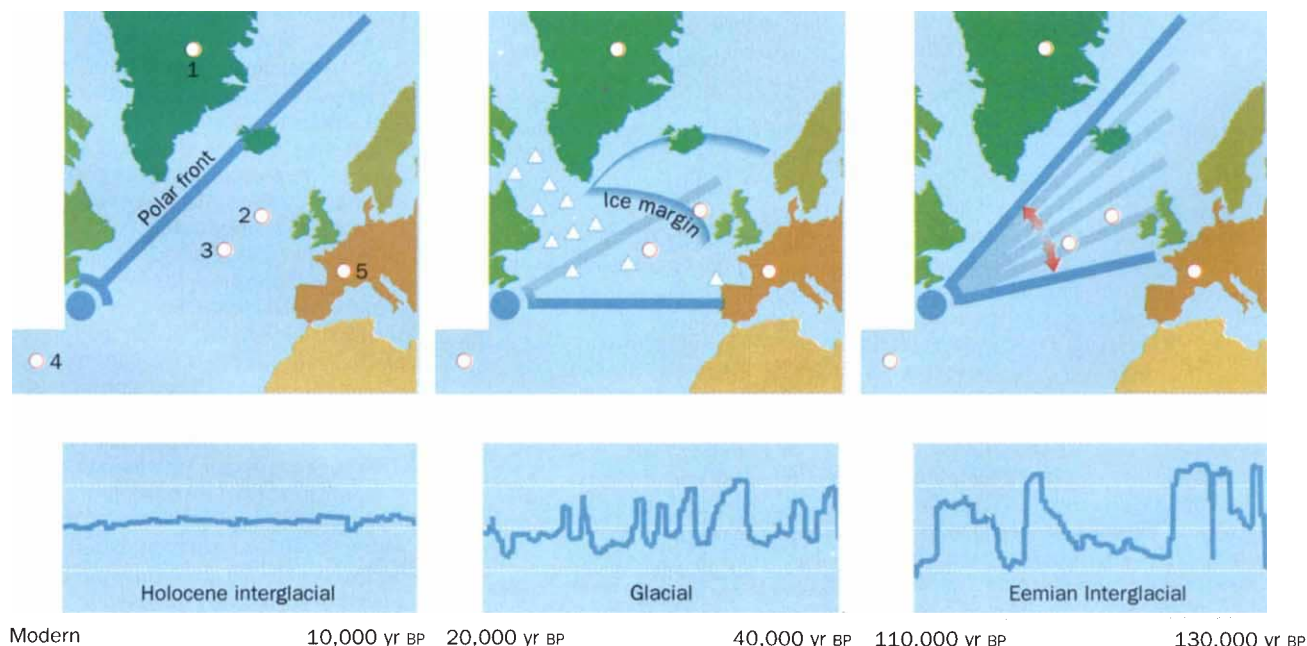
most of northwestern Europe.

During the last glacial period (centre), the 'door' was shut. Warm waters did not penetrate to the north, so the North Atlantic region cooled and continental ice sheets grew. The North Atlantic's thermohaline circulation was much weaker than today. The ice-core record for this period shows rapid climate oscillations thought to be linked to iceberg surges (triangles) coming from the North American 'Laurentide' ice sheet. Quasi-periodic ice-sheet collapses resulted in alternating maximum and minimum rates of iceberg and meltwater discharge to the North Atlantic⁶, which destabilized the glacial mode of circulation to the extent that the polar front door opened, producing intermittent spurts of enhanced thermohaline circulation.

For the peak-interglacial Eemian

period (right), the record from the Greenland Ice-core Project GRIP, though not from the sister core GISP2, implies large-scale and rapid climate oscillations. If the North Atlantic's thermohaline circulation was involved, this would imply rapid retreats and advances of polar water masses which would have resulted in fast shifts of the polar front. The new marine records^{1,2} imply that this conceptual model may hold for the wider interglacial stage 5. For the peak interglacial Eemian, they suggest rather that surface water conditions and deep-water flow in the North Atlantic were stable; but large-scale fluctuations in the Eemian section of a terrestrial pollen record (ref. 9; labelled 5) might be the elusive terrestrial analogues of the climate instabilities seen in the Greenland ice-core record.

R. Z.



the North Atlantic during the early phase of ice-sheet collapse, whereas in the later stages when the ice margin had retreated further inland, iceberg and meltwater discharge ceased, thus allowing in its place an enhanced inflow of warm, salty subtropical waters.

This elegant concept neatly explained the early evidence from the ice cores. But meanwhile, the GRIP members had drilled deeper and published some still more disturbing news^{7,8}. During the last interglacial, in the period known as the Eemian, when there were no great ice sheets around, climate instabilities appeared to have occurred which were at least as strong as those reported for the glacial period. This time, though, the GISP2 record contradicted the GRIP results⁵, and doubts arose as to whether the Eemian section of the GRIP core was distorted by anisotropic shear caused by ice-sheet flow.

One thing seems certain: if the GRIP ice-core record is correct, the Eemian climate jumps should have left their imprint in other climate records such as marine sediment cores. Both Keigwin *et al.* and McManus *et al.* have looked at deep-sea sediment cores drilled in the North Atlantic, concentrating on what they could tell us about climate during the last interglacial period (marine isotope stage 5, 135,000–65,000 years ago). This interglacial was punctuated by two colder 'stadials' when there was more continental ice, and three 'warmer' interstadials when there was less. The warmest of these, about 120,000 years ago, was the Eemian.

But the specific aims of the two groups were rather different. Keigwin *et al.*¹ use records of carbonate content and carbon isotopes which are indirectly linked to the deep ocean's chemistry rather than to surface conditions. These provide proxies for the rates at which deep waters are formed at high latitudes and are being pumped through the North Atlantic. Significant variability is seen in the records, suggesting that deep-water circulation was highly variable throughout the last interglacial isotope stage 5. This picture is corroborated by the data provided by McManus *et al.*², who extend records of abundances of polar planktonic foraminifera (marine microorganisms which live close to the sea surface) back much further than those of Bond *et al.* They show that surface water variability in the subpolar North Atlantic was equally high in response to retreats and re-advances of polar waters.

Within the limitations of the age models for the sedimentary records, the surface and deep-water profiles generally correlate with the climate profiles from the Greenland ice cores. So it seems that the conceptual model of a close link between the North Atlantic's thermohaline circulation and climate variability applies also for

the wider interglacial stage 5. But for the Eemian peak-interglacial period (isotope sub-stage 5e), neither data set shows anything like the variability we would expect from that seen in the Eemian section of the GRIP ice-core record. The records from the subpolar North Atlantic which are closest to the Greenland ice sheet are smooth and stable, and those from the subtropical North Atlantic show no more variability than is seen in other marine records throughout the world ocean. One may argue that, as at present, the Eemian ice sheets were simply too small to trigger occasional iceberg surges and meltwater discharge, thus making climates in the North Atlantic region stable.

The work of both Keigwin *et al.* and McManus *et al.* supports this intuitive argument, in that the surface and deep-water variability seen in their records starts immediately after the Eemian peak-interglacial. This was when larger volumes of continental ice first began to accumulate in high latitudes even though climate conditions were still in an interglacial mode. Keigwin *et al.* have in fact compiled terrestrial palaeoclimate data which indicate that post-Eemian continental climates as far south as California responded to the brief convulsions of the North Atlantic traced in their data. During the Eemian peak-interglacial, however, none of the terrestrial records shows climate signals comparable to the ice-core signals.

With these data, it might seem that the book is closed on Eemian climate instabilities. But there is an intriguing footnote to the story. In Central Europe, a French team believes it has indeed found evidence for Eemian climate instabilities. Thouveny *et al.*⁹ have obtained pollen records of climate from lakes in France which reveal an astonishing similarity both in structure and timing to the GRIP ice-core record. Details will be revealed in a forthcoming issue of *Nature*; but terrestrial palaeoclimatologists may have an ace up their sleeve with which to solve the current stalemate between the marine and ice-core teams. It may well be that for some reason the Eemian climate jumps left the marine system in the North Atlantic region untouched, thus handing the book over to the terrestrial community to advance our understanding of the non-conservative behaviour of our climate system. □

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Plane with fire

EVERY solid surface exposed to air acquires a monolayer of gas molecules, including those of oxygen. Charcoal can absorb enough oxygen to ignite spontaneously. Charcoal saturated by oxygen can even be exploded. Daedalus has been generalizing from these facts. He reckons that on a smooth combustible surface with an oxygen monolayer, a novel 'surface explosion' could be transmitted: the burning of surface molecules in that monolayer. Once initiated, it should flash over the surface with a feeble bang.

Such a blast would be propagated by Rayleigh surface waves. The burning of a single monolayer provides only limited energy, but since the resulting shock can spread out in just two dimensions it should still have ample power density to keep itself going. The small release of heat would be absorbed almost instantly by the bulk material.

Most plastics, and indeed all hard combustible materials that adsorb oxygen, should be surface-explosive. They could be set off by an intense local laser pulse, coupled with a sideways kick from a powerful piezoelectric transducer. There will be a surface flash and a weak bang. A millisecond or so later, the surface would have taken up another monolayer of oxygen and could explode again.

The repeated explosion of a surface could make a novel stroboscopic lamp. It would also be the ultimate in precision machining. A plastic lens or mirror could be precisely shaped by the explosion of successive monolayers of its substance. With a cylindrical workpiece, the explosion might even run continuously round it, lathe fashion, generating a loud howl at its rotation frequency.

Surface explosions may, however, be most useful as cleaning agents. Debris, dust and bacteria would literally be blown away, and possibly burnt up in the process; the surface would be cleaned and sterilized instantly. Better still, the surface need not be tidied first. Objects resting on a surface seldom displace the oxygen monolayer, so the explosion would travel beneath them. Hospital surfaces, kitchen tables with food still on them, grimy painted walls, bookshelves loaded with books, all could be intimately and instantly cleaned for the loss of one monolayer of their substance. Daedalus even began to dream up a human instant-cleaning process. One bang, and you would be tingling-clean from top to toe without even taking off your clothes. Sadly, human skin is too soft and lossy for a surface explosion to propagate. Soap and water will be with us for a while yet.

David Jones

Cumulus clouds and UV-B

SIR — It is well known that clouds can attenuate solar ultraviolet-B (UV-B) radiation at the surface¹. Less well known is that scattering from the sides of cumulus clouds can enhance total (global) solar irradiance by 20% or more over the maximum solar noon value². This phenomenon occurs in the UV-B³, and here we report brief (about 0.5-hour), biologically significant increases.

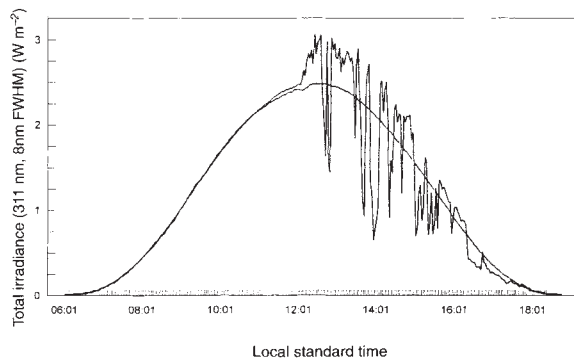
During the 1994 Hawaii Ultraviolet Survey, the first in a planned annual series of observations, 10 miniature UV-B (310 nm) total-sky radiometers were placed in lava at elevations ranging from sea level to 3.4 km across the island of Hawaii from 11 to 24 June 1994. Two pairs of 940- and 1,020-nm total sky radiometers were used to detect the presence of total water vapour and cirrus clouds during the entire study, one pair at the Mauna Loa Observatory (elevation 3.4 km) and

cloud-free day. The integrated increase in UV-B caused by cumulus clouds was compensated for by blockage of the solar disk by clouds. Cumulus clouds arrived over the observatory at 12:01 local time, and from then until 16:01 local time the mean total-sky UV-B was only -0.7% below that on the cumulus-free day.

The significance of these observations is that the two episodes of increased UV-B occurred at or near solar noon and their duration was sufficient to reduce the time required for erythema (reddening) of exposed human skin by at least 15% below that expected under an identical ozone column and a clear sky. Similar enhancements of UV-B caused by cumulus clouds were observed in August 1994 at Seguin in south-central Texas, but only on haze-free days.

One approach to treating the effects of clouds is to estimate the attenuation pro-

Total-sky UV-B radiation at 310 nm at Mauna Loa Observatory on 19 and 22 June 1994 (smooth and jagged lines, respectively). Increases in UV-B on 22 June are caused by reflection from cumulus clouds. Decreases are caused by clouds blocking the solar disk. Observations are by 1 of 10 miniature ($1.7 \times 5 \times 5$ cm) UV-B radiometers developed for this survey. Each instrument can accept interchangeable, water-resistant, plug-in irradiance probes (1.9 diameter $\times$ 4 cm). UV-B is detected by a narrow bandpass (7-nm) interference filter with less than 2% UV-A leakage. A near-cosine response is provided by a snap-on plastic (Teflon) diffuser. The photodiode amplifier design is similar to one described elsewhere⁴. Each instrument includes a programmable microcomputer (Onset Computer Corp.) capable of storing 1,800 samples of peak or average irradiance during intervals ranging from 0.5 s to several hours. During the 1994 Hawaii UV-B Survey, peak and average observations of UV-B at 2-s intervals were also made at various depths under water. These observations and the design of the new radiometer will be described elsewhere.



the other at various sites near sea level.

On several days and at various locations, cumulus clouds increased solar UV-B by 25% or more over the expected value. The figure shows the total-sky UV-B at Mauna Loa Observatory on a day free of cumulus clouds and a subsequent day when cumulus clouds appeared shortly before solar noon (12:22 local time) and persisted through the afternoon. Dips in irradiance occurred when a cloud blocked the solar disk. Significant increases in irradiance occurred when cumulus clouds were near the Sun. The mean increase over the expected irradiance from 12:13 to 12:34 local time was 16.8% (peak, 23.6%). The mean increase over the expected irradiance from 12:51 to 13:25 was 16.5% (peak, 22.8%). The highest cloud-induced increase during midday occurred at 14:51 when total UV-B was 29.8% above that on the previous

provided by a cloudy sky using regression models based on readily observable properties such as fractional cloud cover. This method is most similar to that used to produce the US ultraviolet index made available to the public on a daily basis since 28 June 1994. A deficiency of this method is that it cannot produce an irradiance in excess of the value for clear skies.

Enhancement of UV-B by cumulus clouds is not necessarily evident in measurements made by commonly used time-integrating UV-B radiometers and especially in predictions based solely on satellite ozone measurements. Therefore,

the general public should be advised that cumulus clouds near the Sun can significantly intensify solar UV-B, particularly during summer and especially when both the Sun and cumulus clouds are near the zenith.

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Calcium signals in neurons

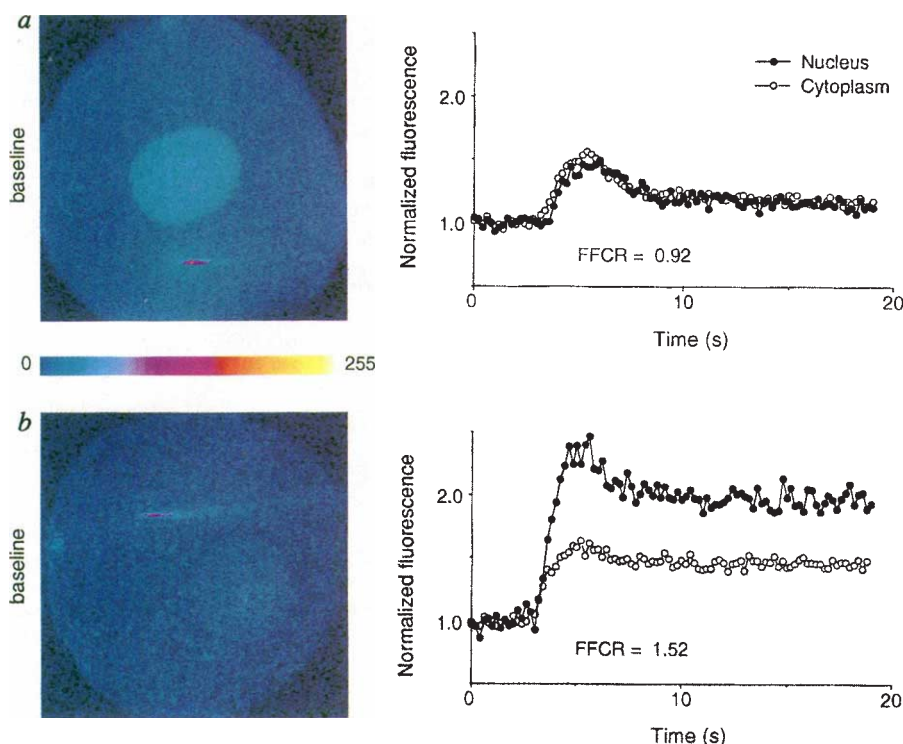
SIR — Al-Mohanna *et al.*¹ state that previous reports²⁻⁵ of nuclear amplification of Ca^{2+} in neurons are due to measurement artefact, and claim that nuclear Ca^{2+} signals are actually lower than cytoplasmic Ca^{2+} signals upon depolarization. Here we discuss the important question of whether or not nuclear Ca^{2+} might be autonomous by examining a technical problem which could undermine this conclusion¹.

A key technical consideration is whether disruption of the cell membrane with a patch pipette or a sharp micro-electrode can damage the cells under investigation and so cause an increase in Ca^{2+} over the normal baseline. The data of Al-Mohanna *et al.* indeed show an important effect of Ca^{2+} -indicator dye sequestration, but because the physiological status of their N1E-115 neuroblastoma cells and adult rat dorsal root ganglion (DRG) neurons was not evaluated, it is possible that the cells were either injured or stimulated, or had their Ca^{2+} signals activated as a consequence of micropipette loading of dye.

To support their data, Al-Mohanna *et al.* present a simple model of centripetal Ca^{2+} diffusion which does not take into account Ca^{2+} -induced Ca^{2+} release (CICR), the activity of Ca^{2+} -binding proteins, and the limited diffusion of Ca^{2+} in cytosol⁶. It is possible that CICR may have been activated in their neurons; an initial small influx of Ca^{2+} can be substantially amplified by CICR in neurons^{3,7}, including adult rat DRG neurons⁸. These elevated Ca^{2+} signals can last for tens of minutes⁹, offsetting baseline Ca^{2+} signals at rest.

We tested the possibility that micropipette disruption of the cell membrane might alter baseline levels of Ca^{2+} by non-disruptively loading adult rat DRG neurons with Fluo-3AM and confocally imaging the neurons before and after sharp microelectrode penetration ($n = 12$). On disrupting the cell membrane with the micropipette, overall Ca^{2+} signals increased by 103%. These results suggest

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Baseline nuclear and cytoplasmic Fluo-3 fluorescence change ratios (FFCR) in neurons with large (a) and small (b) elevated Ca^{2+} signals after disrupting the cell membrane with a sharp micropipette (the pipette tip is the small bright spot in both confocal images). Normalizing fluorescence changes during depolarization to these baselines revealed a proportionally equivalent increase in nuclear and cytoplasmic signals for the neuron in a, and a 1.5-fold greater increase in nuclear signals for the neuron in b.

that the Ca^{2+} signals of the neurons studied by Al-Mohanna *et al.* were elevated at baseline as a consequence of the dye-loading technique.

Using sharp microelectrodes to inject Fluo-3 into DRG neurons, as did Al-Mohanna *et al.*, we detected apparent amplification of nuclear Ca^{2+} signals only in neurons minimally injured by the impalement. The figure shows normalized fluorescence (fluorescence change ratios¹) in the nucleus and cytoplasm of two neurons that were impaled with sharp microelectrodes and allowed to fill with 1 mM Fluo-3 for up to 4 minutes (to minimize dye sequestration artefact¹), then depolarized during confocal imaging. Both neurons maintained physiological resting potentials (less than -55 mV); however, the neuron shown in a in the figure took about 10 seconds to acquire its resting potential, indicating that there had been a slight injury upon impalement, whereas the neuron in b stabilized its resting potential immediately after penetration. After depolarization, the fractional fluorescence change ratio of this neuron indicated an increase in Ca^{2+} 1.5 times greater in the nucleus than in the cytoplasm. In contrast, the neuron in a responded as in the Al-Mohanna *et al.* report, with a somewhat reduced increase in nuclear Ca^{2+} fluorescence compared with the cytoplasm. The most obvious difference is that the neuron in a had

proportionally higher nuclear fluorescence at baseline. Altogether, eight of the neurons we examined had elevated baseline signals and gave responses similar to that in a (no apparent amplification), and five neurons had moderate baseline signals and gave responses similar to that in b (apparent amplification).

Thus, in our opinion, the use of microelectrodes to introduce Ca^{2+} indicator dyes into neurons can cause problems for evaluating baseline nuclear Ca^{2+} signals, and we believe that the important question of whether nuclear Ca^{2+} amplification indeed occurs remains unanswered.

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AL-MOHANNA *ET AL.* REPLY — In our hands neither temporary cell impalement with a sharp micropipette nor patch clamping permanently raises the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). For instance, N1E-115 cells injected with Fura-2 by micropipette had an average $[\text{Ca}^{2+}]_i$ of 88 nM (ref. 10), whereas $[\text{Ca}^{2+}]_i$ in patch-clamped cells was 95 nM (ref.

11). Rand *et al.* state that a bright nucleus in a cell injected with calcium-green or Fluo-3 indicates that $[\text{Ca}^{2+}]_i$ is high. This is not so: nuclei fluoresce more brightly because there is more dye there. Careful work from 1975 onwards¹² has shown that after equilibration the gross concentration of small polar solutes is higher in the nucleus, because the significant volume of the cytoplasm that lies within membrane-bound organelles is inaccessible to the solute. Records, such as that in b of Rand *et al.*'s figure, in which the nucleus is not brighter, must be in error, as are the $[\text{Ca}^{2+}]_i$ changes calculated from such records. There are two likely sources of error: one is uptake of dye into organelles, as discussed in our paper¹; the other is incorrect correction for autofluorescence, as discussed by O'Malley¹³.

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Male suckling

SIR — The fascinating report of mammary development and signs of secretory activity in male Dayak fruit bats by C.M. Francis *et al.* in Scientific Correspondence (*Nature* **367**, 691; 1994) is a splendid illustration of important questions being raised by investigation of whole animals in their environment. The question in this case is: do male fruit bats suckle their young? Further studies are essential because milk secretion without suckling would be a curious biological phenomenon in a wild mammalian population, but one that might be explained by phytoestrogens in these frugivorous bats. Suckling, and the physiological investment of lactation, by a male of any species as a normal part of its life history would be an important biological phenomenon.

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Weapons of bewilderment

James Jasper

One World or None: A History of the World Nuclear Disarmament Movement. Vol. 1 — The Struggle Against the Bomb. By Lawrence S. Wittner. *Stanford University Press*: 1993. Pp. 456. \$24.95.

SOON after the Second World War, Enrico Fermi's sister wrote to him from Italy: "Everyone is talking about the atomic bomb. . . All are perplexed and appalled by its dreadful effects, and with time the bewilderment increases rather than diminishes." Ever since then, despite ebbs and flows, this bewilderment has increased. Nuclear dread has become a prominent feature of the modern world, periodically leading to enormous public demonstrations against atomic weapons. Indeed, opposition to these weapons has been around longer than the weapons themselves, dating back to 1903, when a British chemist warned that radioactivity could lead to explosives "inconceivably more powerful than any we know of". In 1913, H. G. Wells published *The World Set Free*, in which a war fought with "atomic bombs" was so devastating that its survivors decided to form a world government to avoid future wars. So striking to the imagination is it, that dread of the bomb preceded the bomb itself.

Lawrence Wittner describes nuclear policies and, especially, public responses to them, from Wells's novel in 1913 through to the end of Harry Truman's presidency of the United States in 1953. The first of three projected volumes on the movement — or, more precisely, movements — for nuclear disarmament, *One World or None* has appropriately universal coverage, from major players such as the United States and the Soviet Union to smaller countries such as Denmark and New Zealand. It is smoothly written, generally accurate and amply documented (for example, in the bibliography Wittner refers to 37 movement periodicals and 100 collections of private papers). Almost half the book describes the non-aligned movements that developed from 1945 to 1951, another quarter the communist-led movements of the same period, and another quarter the consequences of the movements (despite Wittner's best efforts, he finds precious little evidence of benefits).

Although Wittner surveys region after region, including Eastern Europe, the Third World, the countries defeated in the Second World War, as well as the former Allied nations, the story is similar for most of them, making the book seem repetitive even when it is not. Although the bombings of Hiroshima and Nagasaki in 1945 were widely acclaimed as a means to end the Second World War, nuclear weapons

became more controversial in the following five years because of US government secrecy, the emerging arms race, development of the hydrogen bomb and Truman's loose talk about using nuclear weapons in Korea.

Opponents of nuclear weapons faced a challenge: what position to take on the

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Politics and pacifism: CND handbill, 1963.

nation-state and war in general. Pacifists, having been discredited during the war (Hitler's regime, after all, was cogent evidence in favour of just wars), were unable to take the lead in antinuclear mobilization. Besides, they had broader goals than fighting one form of weaponry, no matter how destructive. All wars and weapons were wrong, and the only way to stop nuclear weapons was to stop wars. One-worldists, who favoured a strong international government to curtail the competitive urges of national governments, became more popular as a result of the war. Nuclear weaponry made their crusade more urgent, suggesting that "Or None" be added to their slogan. Also, there were communist-led peace organizations preoccupied with US weapons but excusing Soviet ones as guarantees of world peace; they spent much of their time attacking noncommunist peace groups as myrmidons of imperialist warmongers. Finally, nuclear scientists often formed the best organized and most creditable

groups to question nuclear weapons.

For obvious reasons, these assorted groups made little headway with national governments. Once a government and its military had the bomb, the most powerful tool in their arsenal as a nation-state, they were quite unwilling to give it up. When, after 1950, anti-communist crusades got into high gear, the antinuclear movement faded rapidly.

Although Wittner is concerned to present facts carefully and not to interpret them, his book lays out several sharp contrasts and paradoxes. One is that innumerable people have been aroused to protest against nuclear weapons in the past 50 years, perhaps more than joined any other political cause, yet their efforts have had almost no influence on government policies. Only governments without these weapons favoured policies to curtail them. From being merely a weapon, atomic bombs became the centrepiece of world diplomacy, especially for the two so-called (and they are called this because of their large nuclear arsenals) 'superpowers'. The mindset of international strategists remains alien to most people.

Equally unsettling is the gap between the mental worlds of government officials and those of the scientists who worked on the bomb. Throughout the history of both civilian and military nuclear energy, scientists and technicians have usually tried to move slowly and work out the flaws of their risky technologies, whereas politicians, the military and even regulators have pushed technologies into use as quickly as possible. This tension began as early as the Manhattan Project, whose director, General Leslie Groves, was constantly suspicious of the scientists working under him, especially when they expressed any doubts; the Nobel prizewinner Fermi, for example, was dismissed as a "wop". Surveillance by the US Federal Bureau of Investigation, removal of security clearances and even dismissal from their jobs awaited scientists who expressed reservations.

The symbolic power of the bomb matches its explosive power. For diplomats, presidents and generals, its lure was almost magical. For many scientists, including those who had helped to develop it, this magic was diabolical. Fear, confusion, national pride and — occasionally — indifference can all be found among public attitudes. Atomic weaponry usually inspires apocalyptic rhetoric, by supporters as well as detractors. Even among its opponents the bomb has meant different things to different groups, but it has always meant a lot. As a result, the awe and bewilderment that Fermi's sister described have never subsided. □

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Directions in time

Peter T. Landsberg

Physical Origins of Time Asymmetry.

Edited by J. J. Halliwell, J. Perez-Mercader and W. H. Zurek. Cambridge University Press: 1994. Pp. 515. £60, \$100.

NOBEL prizewinners are, of course, very clever but nonetheless not infallible. Thus one wrote recently about the book of another: "The chapter on 'Time's Arrows' is a confusing blend of speculation and possibly wrong ideas". In fact, it seems widely agreed that the problems highlighted by the title of the book under review here are largely unsolved, and therefore interesting to discuss. What is the point of discussing solved problems, after all, except in a classroom? These clever 42 people were having real fun (in 35 papers) down there in Huelva, Spain, as the 1991 autumn leaves fell. And they produced a veritable fireworks of ideas in computation, physics and cosmology. Some of the discussion is also recorded, and this adds considerably to the reader's understanding and enjoyment of the book.

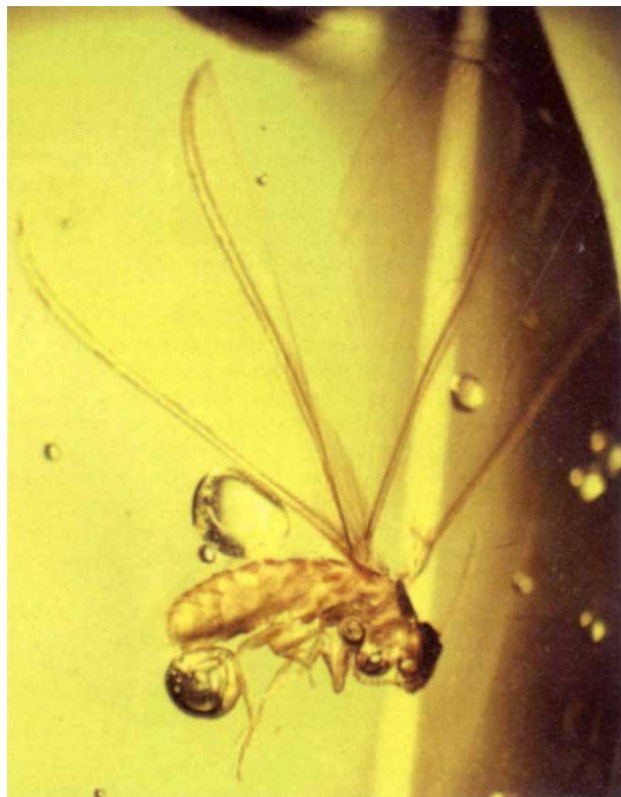
Take, for example, the grand area of "Complexity in the Universe" (C. H. Bennett). The resulting printed questions are surprisingly basic: Is thermodynamic depth a measure of complexity? Does complexity increase like entropy? Is our

Universe deeper than any other conceivable universe? (The answer, I am glad to report, was "I do not know".) Or even: Is a beer can pull-tab as complex as the civilization that produced it? These are of course still matters for discussion. Further, one participant is described later in the book (I hope and believe incorrectly) as that rather rare bird who manages to believe in the existence of "many worlds" while being a solipsist at the same time.

To whet appetites, I now owe readers, particularly physicists, a selection of interesting thoughts gleaned from the book. First, R. Griffiths reminds us that it is incorrect to suppose that the macroscopic direction of time is determined by a small violation of microscopic time-reversibility. Remove a magnetic field from an equilibrating system (which therefore satisfies the second law and violates time-reversibility), and it will still proceed towards equilibrium, even though it is now subject to what is largely time-reversible mechanics. Second, the symmetry in information between past and future, given the present (it is as uncertain where we have come from as where we are going), follows from the assumption that one is dealing with a stationary Markov process; a time-symmetry assumption is not required (T. M. Cover). More obviously, perhaps, the ability of a physical system to act as a computer must degenerate with time, so that a permanent computer is impossible. Third, a well-known fact is put in a slightly

unusual way by P. C. W. Davies when he talks about an "entropy gap", which describes by how much the entropy of the Universe at a certain time lies below the entropy for the equilibrium condition at that time. The expansion of the Universe then increases this gap, whereas irreversible processes such as stellar radiation decrease it. He also remarks that in biology one deals with quality of information (described as "depth"). There is an arrow of increasing 'depth', which, he believes, is not yet understood. Of course, biologists may contend that they do understand it in their own terms — it is called evolution — but the real challenge is to understand the intricacies of evolution in terms of physical principles. Fourth, there is a welcome, albeit elementary, reminder from J. L. Lebowitz that for a few hard spheres in a box one cannot arrange the time-order of snapshots, although this can become possible for a large number of such spheres. Large systems seem to have a more clearly developed arrow of time. Also, Halliwell recalls that an expression for gravitational entropy remains to be discovered. As is well known, this casts doubt on several cosmological speculations. Fifth, R. Omnès challenges orthodoxy by suggesting that not every quantum-mechanical observable is measurable.

An earlier book in this class is *Complexity, Entropy and the Physics of Information* edited by W. H. Zurek (Addison Wesley, 1990), which is based on a 1989 meeting held at Sante Fe, New Mexico. At least 14 participants were common to both meetings. In both cases the proceedings were stimulating but "somewhat diffuse" (*Nature* 349, 376; 1991), and now — editors should really be more painstaking — we do not even have a subject index. This is a pity: for example, the issue of coarse-graining is raised surprisingly often, and an index would enable one to evaluate current views on this topic without reading all 515 pages. I have, however, done this particular job for readers of this review. It is still accepted that coarse-graining is essential, which is satisfying for those who learnt statistical mechanics even before I did, say from R. C. Tolman's book *The Principles of Statistical Mechanics* (Clarendon, 1938). It seems to be accepted that the behaviour of the entropy depends on how this coarse-graining is carried out. This could make the resulting entropy somewhat anthropomorphic, which would be another puzzle. This problem was raised at different times during the meeting by M. Gell-Mann, W. H. Zurek and J. J. Halliwell, and was considered particularly by B. L. Hu. A spirited defence of the view that entropy is for real, and not anthropomorphic, was incidentally given by K. G. and J. S. Denbigh in 1985 (in their book *Entropy in Relation to Incomplete Knowledge* (Cam-



In 1992, George and Roberta Poinar hit the headlines with the news that they and their colleagues had extracted 'live' DNA from an insect trapped in amber about 40 million years old. Last year, they went on to obtain DNA more than 125 million years old, dating from the dinosaur era. In *The Quest for Life in Amber*, the Poinars now tell of the 30-year scientific odyssey leading up to the discoveries. The book, to be reviewed in a future issue of *Nature*, is published by Addison-Wesley on 16 September at \$25.

bridge University Press).

Any book on time asymmetry can justifiably include a more detailed discussion of any of the well-known irreversible processes, because these always imply an arrow of time. But this would not be essential and might lengthen the exposition unduly. I fear that this has been done in the present book by including problems of decoherence in quantum mechanics: that is, the leaking out of quantum coherence from a system into the environment, leading to the emergence of classical behaviour. The 80 pages or so devoted to this topic thus deal essentially with the irreversible processes of quantum measurements. Indeed, B. DeWitt goes on record as saying that the arrow of time has no basic role to play in decoherence. Incidentally, the topic of decoherence can also be studied in the 1989 Santa Fe book.

Perhaps the most striking paper is by J. Barbour, who determined by a strawpoll

that a majority of the participants think that 'time' should be derivable (like temperature) in a truly fundamental theory. He proceeded to indicate non-mathematically an approach that might possibly achieve this; it is of course a little early to judge its success. One might go further and blame the 'accident' of our physiological make-up for the problem we have regarding time, its meaning and its asymmetry. Could, for example, a dematerialized intelligence grasp all developments in a flash and so not need a time coordinate?

The distinction of the participants and the intrinsic interest of the topics discussed make this a book that should be available to all physicists as well as to students of cognate subjects. □

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Telling tails

Robert Gibson

Arena Birds: Sexual Selection and Behaviour. By Paul A. Johnsgard. *Smithsonian Institution Press: 1994.* Pp. 330. \$47.95, £26.75.

Sexual Selection and the Barn Swallow. By Anders Pape Møller. *Oxford University Press: 1994.* Pp. 365. £35, \$49.95 (hbk); £16.95, \$24.95 (pbk);

INTRICATE sexual ornaments such as the peacock's train defy an easy evolutionary explanation. Darwin thought that they attracted mates but was unable to explain why females should find such elaborate structures attractive. More recently the latter issue has become a battleground between adaptive and nonadaptive views of the evolutionary process.

Paul Johnsgard's book is a useful starting point for anyone interested in the plumages and antics of birds that inspired Darwin. Leks — gatherings of males visited by females for mating — have evolved in at least 11 avian families and are associated with extreme polygyny and some of the most elaborate examples of sexual ornamentation. Johnsgard's semi-popular account covers most of the lek-breeding species and some others (such as the bowerbirds and some ducks) with lek-like mating behaviour, and is copiously illustrated with photographs and his own pen-and-ink sketches. The bibliography provides an up-to-date entry into the primary literature.

At first sight, a socially monogamous songbird in which males possess outer tail feathers a fifth longer than females seems an unlikely subject for answering questions raised by the peacock's train. Yet

more has been learned about sexual selection from the barn swallow than from any lekking bird, due in part to the ease with which tail feathers can be lengthened or shortened with scissors and superglue. The story, based on a 10-year study in rural Denmark, is told by Anders Møller in his monograph. Its general interest is likely to rest on three principal results.

First, tail length affects a male's ability to acquire a mate, with many different consequences that influence his reproductive success. Second, females may benefit from mating with long-tailed males by avoiding infestation by ectoparasitic mites that abound on shorter-tailed males. There is also an intriguing indication, obtained by transferring chicks between nests, that females may gain genetic resistance to mite infestation for their chicks by mating with longer-tailed males, although the trait conferring resistance remains a mystery. Finally, tail length has the properties of a Zahavi handicap: long tails are indicative of phenotypic vigour (as shown by relationships between final tail length and both the rapidity and perfection of feather growth) and experimentally lengthened tails impose a mortality cost that falls more heavily on less vigorous individuals, which may explain why honest signalling is evolutionarily stable.

Møller's field studies are remarkable in scope, in the degree to which experi-

mentation has been employed and in the extent to which they confirm *a priori* theory. Collectively they make one of the most complete cases so far for adaptive female choice of a sexual ornament. But how robust are the conclusions?

This is more difficult to answer. One problem is that while marshalling data in support of particular hypotheses, Møller often fails to discuss plausible, though less interesting, alternatives. A recurrent issue is the extent to which differences in annual return rates represent survival as opposed to modulations of site fidelity in relation to previous breeding success (well known from many birds including the barn swallow).

Another is the extent to which resemblance between offspring and parents in characters such as tail length, mite load and survival represents additive genetic effects as opposed to direct parental and environmental contributions. These are not easy matters to resolve, but they critically affect the conclusions. A decision not to burden the text with technical details also robs the reader of sufficient information to evaluate particular results, and sometimes even to understand their origin. (I remain perplexed as to how 23 per cent of 81 unmated males were classified as committing infanticide when only 4 cases of infanticide were observed.) It is a pity that such problems erode confidence in some of the book's more interesting conclusions. □

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UNDERNEATH the arches — barn swallows (*Hirundo rustica*) feeding young.

Surprise surprise!

Joseph Ford

Complexification. By John L. Casti. HarperCollins: 1994. Pp. \$25.00. To be published in the United Kingdom by Abacus on 1 December at £7.99.

WHEN a scientist reviews a book in his area written for a general audience, he first scans the index for reference to his name and works. He then peruses the chapter closest to his heart, checking for coverage and technical accuracy. Finally, he browses through the rest of the book, paying special attention to any novel ways of presenting technical material and clever ways of aiding understanding for the novice, as these are items that might prove useful in his own teaching and writing. Whether or not these remarks are too jaundiced, readers of the following review are advised to bear in mind the biases a scientist wears as permanent equipment. Recall that the scientific reviews of James Gleick's *Chaos*, for example, were cool to hostile, whereas everyday readers kept it on the *New York Times* bestseller list for months, forcing it into innumerable re-printings. So, with the sides now clearly drawn, let the battle begin.

John Casti brings impressive credentials to the task of writing another book for a general audience on complexity. He is not only multi-degreed but also closely linked with the Sante Fe Institute, a recognized hotbed of complexity. Moreover, he has written several earlier books in this genre

that have been well received. The technical coverage in his latest book is extremely broad and he is highly ingenious in finding effective ways to lead the novice gently into technical areas. With all these factors going for it, what could possibly go wrong? In this regard, recall that the first edition of Herbert Goldstein's *Classical Dynamics* was 'lean and mean' and became the text of choice for several decades. But then popularity led to a revised edition all fat and bloated and "sicklied o'er with the pale cast of thought". *Complexification* is not fat and bloated but it does show the strain of Casti's inventing a new slant for reviewing much the same technical material as he covered earlier.

The subtitle, "Explaining a Paradoxical World Through the Science of Surprise", defines the theme of the book. Indeed, mention of the surprise generated by this or that scientific result is common and a section labelled "The Science of Surprise" appears near the end. Yet I can say without equivocation that I had no knowledge of a science of surprise before starting this book and I have none now. But this disappointment was only one of several that gave me the feeling that the book is imbued with too much and too little thought. For example, not only did I find the early chapter on catastrophe tough going, but as I turned the pages I found myself feeling an acute sense of *déjà vu*. Only later did I notice that it was caused by eight sequential, almost identical drawings of the cusp catastrophe.

On the brighter side, Casti introduces a useful section entitled "To Dig Deeper" to which the reader is referred when the text

discussion has reached the limits of popular presentation. This device is on the whole used sparingly, although in the last quarter of the book it becomes annoyingly frequent. The ploy has some of the character of a flash-card section at a sporting event. And I must draw attention to two technical matters. Casti reproduces all but the punch line of Turing's proof of the halting theorem from which he infers the existence of noncomputable numbers; following this questionable manoeuvre, he then offers the Turing theorem without proof. More seriously, Casti has a field day with the word 'random'. 'Random' itself is followed by 'truly random', 'deterministically random', 'pseudorandom' and 'everyday' or 'real-world random'. For the most part, readers are left to fend for themselves in distinguishing between these terms. Moreover, Casti uses his notions of randomness to prove that chaos is only 'apparently' random, with 'apparently' left undefined. His proof will probably generate a great deal of unintended surprise in the minds of many.

Despite my criticisms, this is not a bad book. A reader can learn much from it. But equally, it cannot be regarded as a good book when compared with the competition, including the author's own earlier works. Even the best of writers has a bad day, however; perhaps a future edition of this work will violate the usual rule and provide the surprise of being better than the original. □

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Tiger (c. 1830), one of several watercolour "portraits" of tigers and lions painted by Eugène Delacroix. The picture is taken from *Noble Beasts: Animals in Art*, a gathering of pictures from the National Gallery of Art in Washington, DC. Included are paintings

by Rubens, Boucher, Manet, Renoir, Gauguin, Bonnard, Vuillard and Hopper. Each work is accompanied by a literary excerpt, by authors such as Homer, Milton, Blake, Yeats and Lawrence. Contains 59 colour plates. Bullfinch/Little, Brown, \$24.95.

The ancient regulatory-protein family of WD-repeat proteins

Eva J. Neer, Carl J. Schmidt, Raman Nambudripad & Temple F. Smith

WD proteins are made up of highly conserved repeating units usually ending with Trp-Asp (WD). They are found in all eukaryotes but not in prokaryotes. They regulate cellular functions, such as cell division, cell-fate determination, gene transcription, transmembrane signalling, mRNA modification and vesicle fusion. Here we define the common features of the repeating units, and criteria for grouping such proteins into functional subfamilies.

MANY proteins are built of modules. In some cases the function of a module is known and its presence in a new protein strongly predicts the protein's function. In other cases, the function of common patterns found in proteins across a wide taxonomic range remains unclear. Here we review the properties of a large family of proteins confined to eukaryotes, in which such a conserved unit recurs four to eight times within each polypeptide. The conserved core of the repeating unit—the WD repeat—usually ends with the sequence Trp-Asp (WD). First found in the β -subunit of heterotrimeric GTP-binding proteins (G proteins) which transduce signals across the plasma membrane¹, it has been called the β -transducin repeat², the WD-40 repeat^{1,3} or the GH-WD repeat⁴. We have identified 27 different proteins containing WD repeats (of which about 20 have been at least tentatively assigned a function), defined what constitutes a repeat, and developed criteria to identify such proteins and predict which of them may form subfamilies with similar functions. Finally, we suggest how the repeats may interact with one another to perform a common function.

By searching Genbank, EMBL and SwissProt databases and with a pattern common to a few prototypic WD repeats⁵ and with the regular expression in Fig. 1, we found 57 sequences representing 27 different proteins, with a total of 269 potential repeats. These repeats have a region of variable length, followed by a core of more or less constant length bracketed by two characteristic pattern elements, GH (Gly-His) and WD:

$$\left\{ \begin{array}{c} \text{X}_{6-94} \\ \text{Variable} \\ \text{length} \end{array} \right\} \left[\text{GH} - \begin{array}{c} \text{X}_{23-41} \\ \text{Constant-} \\ \text{length core} \end{array} - \text{WD} \right]^{N_{4-8}}$$

In 82% of the repeating units in which GH and WD (or their unambiguous equivalents) could be identified, there were 27 ± 2 amino acids (maximum range 23–41 amino acids) between GH and WD. In the same repeats, the number of amino acids from WD to the next downstream GH was very variable (6–94 amino acids), although shorter sequences were more common (61% were 11 ± 2 residues long). Most repeating units are 36–46 amino acids long from WD to WD, suggesting that this represents the primordial length to which inserts or deletions were added.

A regular expression describes a pattern in the core region that is common to most WD repeating units (Fig. 1 legend). There is no such consensus in the variable-length region, but within a subfamily (such as G_{β}), the non-core sequences are highly conserved. The pattern suggests a common structure for the core of the WD repeat that includes two turns and three β -strands (Table 1 and Fig. 2). The unit probably goes from WD to WD, as there are many examples of WD-repeat proteins that end at or just after WD (or its equivalent), but none that ends with a clear GH.

The regular expression in Fig. 1 provides an objective way to assign proteins to the WD-repeat family. We define WD-repeat proteins as having at least one unit that matches the expression with zero or one mismatch, and at least one other unit that has three or fewer mismatches (Table 1). Not all the units exactly match the common pattern. When many pattern elements are missing, repeats are recognized only because they appear together with more obvious ones. When we used the expression to search the translated Genbank database, we found no proteins that contained only one repeat unit (that is, one or no mismatches to the

expression), suggesting that the false-positive match rate is near zero. Whenever we found a clear repeat, it was always among at least three other recognizable repeats. If we screened the database with a simpler expression containing only the boxed components in the original (Fig. 1 legend) separated by the number of residues indicated, and required that the simplified expression occur in a protein at least twice, then all matches contained at least four repeats, and satisfied the criteria for membership in the WD-repeat family. The same expression without requiring repetition identified over 200 sequences, only about 60 of which were true WD-repeat proteins. We conclude that the simplified expression contains sufficient information to characterize the WD protein family, provided we require that it be repeated.

If a protein meets the minimal criteria, then we need to determine its relationship to other WD-repeat proteins, and identify true functional homologues. The properties of the largest group of WD proteins whose functions is known, the G_{β} subunits of G proteins, suggest three criteria for full functional homology. (1) The proteins must have the same number of WD repeats. (2) The repeating units at equivalent positions in different polypeptides must be more similar to each other than to any other repeating units (just as the first WD repeat in a human G_{β} subunit is more like the first WD repeat in *Dictyostelium* G_{β} than it is like any other WD repeat in the same human G_{β}). (3) There must be statistically significant similarity in any amino- or carboxy-terminal extensions (excluding monotonous runs of amino acids). So far, these criteria are met by only four groups of proteins: the G_{β} subunits (including the yeast G_{β} STE4) (refs 6–13), the group of neurogenic Groucho-related proteins^{14,15}, the PR55 and CDC55 phosphatase subunit proteins^{16–19}, and a group of proteins including Cblp, RACK1 (a protein kinase C-binding protein) and Arca^{20–23}. A further pair of proteins meet the criteria only partially. RbAp48, a protein that binds human retinoblastoma protein²⁴, and Msi1, a protein that regulates the ability of Ras to activate adenyl cyclase in yeast²⁵, may be functional homologues, because RbAp48 can partially suppress the temperature sensitivity caused by mutations in Msi1. These proteins satisfy criteria 1 and 3, but not criterion 2. The lack of positional equivalence for the WD repeats in RbAp48 and Msi1 suggests that the functions tested so far may depend on the non-WD repeat regions in each protein.

Functional diversity

Is there a common function associated with WD repeats? Table 1 groups the WD repeat proteins according to their functions; all seem to be regulatory and none is an enzyme. Some, like SEC13, a yeast protein involved in regulation of vesicular traffic²⁶ and RACK1, are entirely made up of repeating elements. Most, however, have extensions, and some have long inserts in the non-core region. It is often unclear which of the protein's functions are due to the WD repeats and which to the amino- and carboxy-terminal extensions, but in a few proteins WD repeats are fused to a known functional domain. For example, a short sequence in the carboxy-terminal extension of PLAP activates phospholipase A_2 ²⁷. Similarly, the amino-terminal extension of the G_{β} subunit interacts with a small protein, the 7K–8K G_{γ} subunit²⁸ which is essential for the folding of the G_{β} subunit into its native structure and for

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TABLE 1 Structure and function of WD-repeat proteins

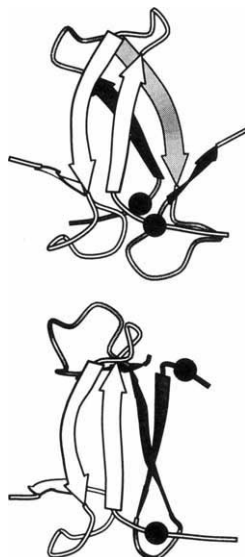
PROTEINS	FUNCTIONS AND INTERACTIONS WITH OTHER PROTEINS*
SIGNAL TRANSDUCTION	
Gβ STE4	Subunit of signal-transducing heterotrimeric G proteins. Regulates G _α subunit receptors, ion channels, adenylyl cyclase, phospholipases C and A, receptor kinases, interacts with calmodulin and phosducin ^{1,6-13,31} . [STE4 = M23982; G _β = P04901, S34348, S61882, M76593, P17343]
LIS1	Subunit of platelet-activating factor acetylhydrolase; mutation in man causes severe brain malformation ^{51,52} . [L13385]
MSI1	Negative regulator of Ras-mediated cAMP increase in yeast, suppresses phenotype of activated <i>ras</i> mutation ²⁵ . [M27300]
PR55	Regulatory subunit of phosphatase 2A ¹⁶⁻¹⁹ . [Q00007, L07583]
PLAP	Phospholipase A ₂ -activator protein ²⁷ . [M57958]
RACK1	Binds activated protein kinase C; ArcA is induced by auxin in plants ²⁰⁻²³ . [X53574, M24193, A33928, Q17526]
RbAp-48	Binds retinoblastoma protein; in yeast partly suppresses heat-shock sensitivity caused by <i>ira-1</i> or <i>ras</i> mutations ²⁴ . [X74262]
RNA PROCESSING	
Cst F	Required for polyadenylation of mammalian pre-mRNA <i>in vitro</i> , associates with subunits of cleavage-stimulation factor ³⁸ . [Q05048]
Mak 11	Needed for double-stranded RNA replication in yeast ⁵³ . [J03506]
PRP4	Associated with PRP6 and other U4U6 snRNP proteins in yeast ³⁵⁻³⁷ . [M26597]
PRP17	Functions in pre-mRNA splicing in yeast (J. Abelson, personal communication). [—]
GENE REGULATION/DEVELOPMENT	
COP1	Represses light-induced morphogenesis when plant is in darkness; zinc-finger domain in N-terminal extension ⁵⁴ . [L24437]
Groucho	Neurogenic locus in <i>Drosophila</i> , nuclear protein ^{14,15,55} . [P16371, L14463, Q04724-7]
HIR1	Repressor of histone gene transcription ⁵⁶ .
TAF _{II} 80	Part of RNA polymerase II transcriptional apparatus, forms a complex with other tightly associated factors (TAFs) ^{39,40} . [S33263]
TUP1	Mutation causes decreased repression of many genes, forms complex with Ssn6-CYC8, a TPR protein ^{41,42} . [M31733]
VESICULAR TRAFFIC	
β'COP	Part of the coatamer multiprotein complex; makes up part of surface of non-clathrin-coated vesicles ^{43,44} . [A44272]
SEC13	Required for formation of mature transport vesicles from the ER membrane ²⁶ . [Q04491]
REGULATION OF CYTOSKELETAL ASSEMBLY/CELL CYCLE	
βTrcp	Expression in yeast suppresses mutations in <i>CDC15</i> , a gene needed for normal anaphase ⁴⁹ . [M98268]
CDC4	Needed for normal spindle polebody formation ⁵⁷ . [X05625]
CDC20	Mutations affect nuclear fusion, may interact with CDC16, a TPR protein ⁵⁹ . [X59428]
Coronin	Binds actin, co-precipitates with actin-myosin complex ⁵⁹ . [X61480]
UNKNOWN FUNCTION	
AAC3	Developmentally regulated ⁶⁰ . [X16524]
MD6	— [S20665]
PC326	— (ref. 61). [S27875]
PWP1	Null mutants grow slowly ² . [M37578]
YCR57c	— (ref. 62). [S19487]
YCR72c	— (ref. 62). [S19487]

The blocks represent WD repeating units for the proteins listed. They are coloured according to their fit to the regular expression in Fig. 1 (black, no misses; light blue, 1 miss; green, 2 misses; white, 3 misses; red, >3 misses). Bars represent extensions at the amino or carboxy termini or inserts between repeating elements. Only one example from each class of proteins is shown, except for rat G_{β1} and STE4, which are both β-subunits of heterotrimeric G proteins and are discussed in the text. Accession numbers shown in square brackets. Groucho is a mammalian homologue of the *Drosophila* Groucho protein. Note that βCOP and COP1 are unrelated proteins with similar names. TPR, tetratricopeptide repeat, a 34-amino-acid motif⁶³. * Since our analysis was completed, the following WD repeat proteins have been identified: p55CDC⁶⁵, Nedd⁶⁶, and unpublished proteins with the following accession numbers: X71810, X72841, L26505, Z33879, Z28021, X75313. Two proteins, VPS15⁶⁷ and SOF1⁶⁸ contain repeating elements similar to WD repeats, but none that matched the regular expression with 3 or fewer misses. These proteins were not included in our analysis. To save space, we have often cited only a recent paper as a guide to primary sources.

region precedes the GH, but other smaller variations occur within the conserved core region. The consensus expression and the common patterns of hydrophobicity (Fig. 1) indicate that the WD repeat may fold with a variable loop preceding the GH, followed

by a β-strand–turn–β-strand–turn–β-strand ending with the WD. Such a small β-structure is unlikely to be stable independently unless stabilized by a ligand (such as a metal ion) or by contact with other WD repeats. If the WD repeats interact with each other

FIG. 2 Model for the conserved core of the WD repeat. The model is based on the high sequence similarity of the regular expression in Fig. 1 with the X-ray structure of the protein at locus 9mt in the Brookhaven Protein Data Bank. These structural assignments were made on the basis of a consensus prediction across our entire data set and by regional 'homologous extension' to three matches of the above regular expression (minus the last two terms and the two identities for H and D). The matched X-ray-determined structure loci in the Brookhaven database were 2mcm, 2rs2 and 4rnt, each in one or two β hairpins. The protein at this locus is ribonuclease T1 (ref. 64). Shown are two alternative packings of a pair of three-stranded subdomains: in both, the two fragments are symmetrically related and appear to have a well-packed interface. Black circles indicate the position of 'H' in the pattern element 'GH' (Fig. 1). The figure only demonstrates the plausibility of such pairwise or multimeric interactions between the WD repeats; other arrangements are also possible.



equivalently, they could form a closed circular structure, but this would predict exposed hydrophobic surfaces. More likely, they could be stabilized as intramolecular dimers or tetramers (Fig. 2).

Some WD-repeat proteins (G_{β} for example) have an odd number of repeats that all match the common pattern well. If the odd repeat never participated in a stabilizing dimeric structure, it should lose its conserved character. Perhaps all repeats participate in pairwise interactions, but under different circumstances. A small shift in the packing could produce a large change in the configuration of the exposed surface. It is not clear whether the conserved core of each repeat binds to any common structure. The fundamental role of the core may be to interact with other cores to

provide a mobile scaffold on which to display the surface specializations. Such mobility would allow a WD-repeat protein to present a changeable surface for protein-protein interactions, and implies that such proteins are capable of significant, protein-induced conformational changes. Different subsets of a protein's WD repeats may moreover be capable of functional interactions with different subsets of the protein's partners.

Where the repeats in a single protein are differentiated, as in G_{β} , Groucho or RACK1 (Fig. 1), each repeat may specify the interaction with different partner proteins. Because each repeat is different, the partner proteins need not have a common structure. Indeed, the G_{β} subunit interacts reversibly with at least six classes of proteins, none of which have any motifs in common. The repeating structure of G_{β} may allow it to tailor its assembly and its exposed surface to fit each one of its protein partners. The same G_{β} subunit functions differently when paired with different G_{γ} subunits; perhaps the various G_{γ} s constrain the packing of the units in G_{β} to favour different states. Amino-terminal extensions or associated proteins may do the same for other WD-repeat proteins.

In general, the more substrates and/or protein subunits with which a particular protein must interact, the greater the number of evolutionary constraints. The conservation of G_{β} and RACK1 is similar to that of the histones (which interact with a wide range of protein and DNA sequences), and the calmodulins (interact with many unrelated Ca^{2+} regulated enzymes). Given the strong conservation of many of these WD protein repeats, we conclude that the functions associated with each were fixed over 1,200 million years ago. The multimeric interactivity apparently bestowed by the WD repeat structure must be significantly older, perhaps dating to the eukaryotic origin. □

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Seismic evidence for a 920-km discontinuity in the mantle

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Analysis of hundreds of seismograms from a seismic array in the Japanese islands reveals a seismic discontinuity at a depth of about 920 km in the mantle beneath the Tonga subduction zone. This discontinuity is also evident beneath subduction zones in the Japan and Flores seas. The discontinuity probably represents either a change in mantle chemical composition or the signature of the subducted slab's garnet layer.

ANSWERS to many key questions about mantle dynamics hinge on the nature of the mantle transition zone¹. For example, the pattern of mantle convection depends strongly on whether the transition from the upper mantle to the lower mantle is related to a chemical or a phase change of the mantle material. In the first-generation models of the Earth's seismic velocity structure, Bullen¹ defined the mantle transition zone (region C) to be the region between the depths of 410 km and 980 km, where the seismic wave velocity changes drastically, though continuously, compared to that above and below.

Detailed seismological analyses in the past 30 years have yielded the current generation of Earth models^{2,3} in which the mantle transition zone consists of two major discontinuities at depths of ~410 km and ~660 km. With the help of the results from high-pressure experiments on rock minerals, we now seem to have a rather universal (although still controversial) view of the nature of the transition zone: the 410-km and 660-km discontinuities are due to the phase transitions of olivine→spinel→perovskite + magnesiowüstite^{4,5}, and the lower mantle starts just below the 660-km discontinuity⁶ with a rather smooth velocity gradient down to near the mantle-core boundary^{2,3}. There exists however, appreciable evidence suggesting that the velocity gradient changes in the lower mantle. Summarizing earlier work⁷⁻¹¹, Johnson¹² concluded that most studies had identified change of velocity gradient in the depth ranges of 900–1,000, 1,200–1,300 and 1,900–2,000 km. Some of the recent studies have also identified reflected or converted seismic waves from those depth ranges, suggesting a possible presence of seismic discontinuities in the lower mantle¹³⁻¹⁶.

In the past few years, signal enhancement techniques applied to a large number of seismograms have been successful in identifying many reflected or converted phases at seismic discontinuities in the upper mantle, helping us to obtain a better picture of the dynamic state of the Earth's deep interior¹⁷⁻²¹. In this paper, we use a large number of seismograms from Japanese stations to show that there is a discontinuity at a depth of ~920 km in the mantle. This discontinuity may actually represent the bottom of the transition zone and thus be the top of the lower mantle.

Signal enhancement with J-array

The J-array is a large-aperture short-period (1 Hz) seismic array across Japan which began recording in April 1991²². It combines several regional seismic networks developed under Japan's national programme for earthquake prediction. With an aperture of 3,000 km in length by 300–500 km in width, it covers the entirety of the Japanese islands (Fig. 1). With more than 300 high-quality short-period vertical-component seismographs, the J-array is ideally suited for analysing the weak signals of reflected or converted waves associated with mantle discontinuities.

In Fig. 2, we indicate the type of rays we wish to observe in the P-wave coda (the portion of records after the arrival of the

direct P wave) if discontinuities are present just below or above deep earthquakes. The notation p_dP (or S_dP) denotes a wave that propagates upward (downward) from the source as a P wave (S wave) and is subsequently reflected as (converted into) a P wave at a mantle discontinuity located at depth d km from the Earth's surface. In contrast to reflected waves, which have incident angles that are shallower than the direct P waves, waves converted from S to P (S_dP) are received at incident angles that are steeper than the direct P wave, as they have smaller values of slowness (the reciprocal of apparent velocity). We use S–P converted waves to constrain the depths of discontinuities. The travel time difference between S_dP and the direct P wave is a simple function of the vertical distance between the source and the mantle discontinuity for a laterally homogeneous structure. Therefore, the depth of the discontinuity can be constrained using estimates of the travel-time difference, assuming we have reasonable estimates of the source depth and that lateral inhomogeneity is relatively insignificant.

Data analysis. We use all earthquakes with depths greater than 500 km that occurred in the Tonga subduction zone and were

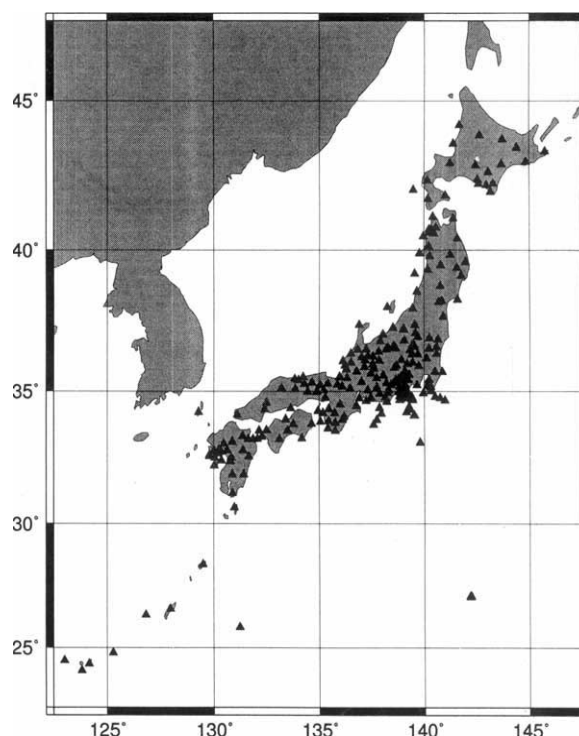


FIG. 1 Location of the J-array stations. The J-array's 'J' comes not only from 'Japan', but also from the shape of the Japanese islands.

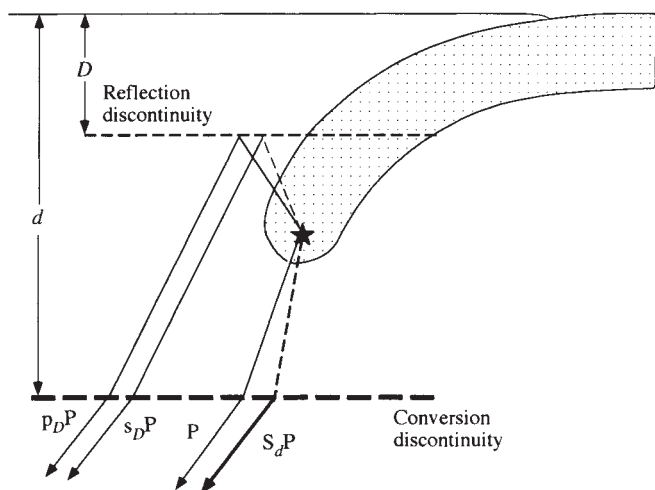


FIG. 2 Typical geometry of seismic rays used in this study to investigate transition zone discontinuities. Lower-case (p, s) and upper-case (P, S) letters are used for waves that initially propagate upward and downward from the source (star) respectively. Solid and broken lines indicate P and S waves respectively.

recorded by the J-array stations. There are a total of seven such events (for the events occurring in 1990 before the operation of the J-array, we use the data of the regional network of the Earthquake Research Institute of the University of Tokyo, which covers the central part of Japan). The epicentral distances are in the approximate range 65° – 77° . We re-determine the hypocentral depths from handpicked pP phases using J-array data and globally distributed broadband data (Table 1). For the case of an unambiguous pP arrival, the source depth variance is as small as 3 km. In general, an accuracy of ± 10 km in hypocentral depth estimate is expected.

Figure 3a shows an example of the J-array recording of a deep earthquake in Tonga. Because individual records are invariably contaminated by near-receiver reverberations and other noise, it is very hard to identify later-arriving phases that are the signature of mantle discontinuities. However, by stacking individual records the signal-to-noise ratio is significantly improved, facilitating the identification of later-arriving phases¹⁸. Seismograms with favourable signal-to-noise ratios are selected and processed with a 2-s low-pass filter. The seismograms are subsequently aligned such that the maximum amplitude of the direct P wave, which is normalized to unity, occurs at time zero (when necessary, the polarity of the seismogram is reversed). Approximately 40–120 station records with a window of 150 s are selected for stacking.

For an incoming wave with an apparent velocity v (or an equivalent, slowness p), the time lag τ_j for the j th station is

FIG. 3 a, Example of a portion of a seismic section for an event occurred in Tonga. Theoretical travel times of the iasp91 model for major expected bodywave arrivals are indicated by straight lines. Note that except for P and pP phases, other phases are difficult to identify in individual traces. b, A colour contour map of the stacked section of the seismograms shown in Fig. 3a. The slowness assumed for each stack is varied with respect to that of the direct P arrivals (defined as zero) in increment of 0.1 s deg^{-1} within the range of $\pm 3 \text{ s deg}^{-1}$. The resulting 61 stacked waveforms are subsequently converted to amplitude envelopes using the Hilbert transform. The maximum amplitude is chosen from all 61 stacked traces and is used to normalize the traces in a unit of decibel. The final result is indicated by a colour contour map in which 'hotter' colour clusters represent greater energy and may represent possible phase arrivals. Note that many later phases between P and pP are present. Major phases are identified by arrows.

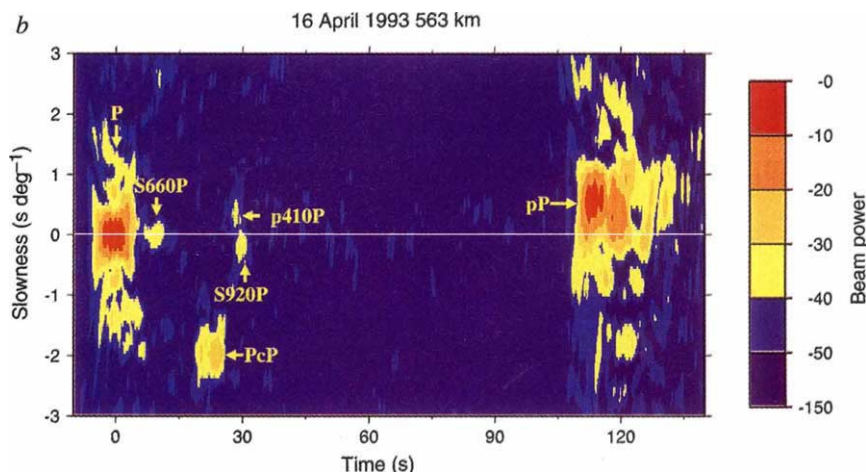


TABLE 1 Earthquakes and observed parameters

Date (yr mo d)	Time (h min s)	Lat.	Long.	Depth (km)†	Region	Mag. mb	'660-km' (T P D)*	'920-km' (T O E D)†	S ₆₆₀ P/P (O C)§	S ₉₂₀ P/S ₆₆₀ P (O C)§
1990 06 08	15 05 10.3	-18.70	181.10	518.	Tonga	5.9	13.5 -0.05 660.	35.0 -0.10 -0.14 920.	0.080 0.068	0.43 0.44
1990 07 22	09 26 18.0	-23.48	179.93	535.	Tonga	5.8	10.5 0.10 655.	-0.13	0.270 0.124	0.27
1993 08 07	17 53 28.5	-23.60	179.10	553.	Tonga	5.9	13.8 0.00 710.	31.8 -0.12 -0.12 925.	0.080 0.117	0.38 0.18
1991 12 03	10 33 42.0	-26.31	178.57	559.	Tonga	5.8	14.4 -0.10 730.	30.5 -0.10 -0.11 922.	0.100 0.163	0.25 0.21
1993 04 16	14 08 40.0	-17.40	181.10	563.	Tonga	5.9	9.7 -0.05 670.	31.1 -0.10 -0.13 925.	0.095 0.110	0.74 0.64
1992 08 30	20 09 06.9	-17.74	181.23	565.	Tonga	5.9	12.8 -0.05 710.	28.6 -0.12 -0.13 900.	0.021 0.083	0.95 0.24
1991 09 30	00 21 47.5	-20.88	181.41	595.	Tonga	6.3	8.0 -0.05 685.	25.7 -0.10 -0.11 900.	0.083 0.081	0.63 0.34
1993 01 19	14 39 26.4	38.57	133.52	434.	Japan Sea	6.0	20.0 -0.08 660.	43.8 -0.10 -0.13 943.	0.055 0.246	0.42 0.22
1991 06 07	11 51 27.6	-7.26	122.57	543.	Flores Sea	6.2	18.0 -0.10 720.	39.0 -0.28 -0.27 945.	0.070 0.096	0.31 0.34

* T, arrival time (s); P, slowness (s deg⁻¹); D, Depth (km).

† T, arrival time (s); O, observed slowness (s deg⁻¹); E, expected slowness (s deg⁻¹); D, depth (km).

‡ Depth from pP-P.

§ O, observed amplitude ratio; C, calculated amplitude ratio.

$\tau_j = D_j/v = D_j \cdot p$, where D_j is the epicentral distance of the j th station minus the epicentral distance of the centre of the array. Let x_{ij} represent the amplitude at the j th station at the i th time for the case with K stations. We use N th root stacking²³; for a given slowness p , an N th root stack, $y(p)$, is given by

$$y_i(p) = R_i(p) |R_i(p)|^{N-1} \quad (1)$$

where

$$R_i(p) = \frac{1}{K} \sum_{j=1}^K \text{sign}(x_{ij} + \tau_{j,i}) |x_{ij} + \tau_{j,i}|^{1/N}$$

N can be assigned any value depending upon the signal quality. When $N=1$, equation (1) reduces to the usual linear slant stack¹⁸; here we use $N=2$. Figure 3b depicts a representative example of an N th root stack for the records shown in Fig. 3a.

The N th root stack analysis has two advantages over examining individual seismograms: (1) the signal-to-noise ratio is improved significantly, and the later-arriving phase S_dP is therefore clear enough to be readily identified. Although many later arriving phases can be observed in linear stacks with $N=1$, by choosing $N=2$, small signals show up more clearly; (2) not just the arrival time but also the slowness of a particular phase is obtained, which aids correct identification of the phase. As described above, an S_dP phase is characterized by a smaller value of slowness than that of the direct P wave, which effectively eliminates the possibility of misidentification of direct P arrivals due to aftershocks. Slowness thus plays a critical role in the identification of later phases.

The 920-km discontinuity identified

In Fig. 4a, N th root stacked traces of the seven deep events are plotted with respect to source depth. In this figure, many late-arriving phases are evident and the most prominent phase, which is denoted by a closed circle, is identified as the S-P conversion wave associated with the 660-km discontinuity ($S_{660}P$). The $S_{660}P$ arrivals have a maximum delay of approximately 6 s from the theoretical time, which corresponds to a 60-km deepening of the 660-km discontinuity^{18,20} (the result of our analysis of the geometry of the 660-km discontinuity in Tonga will be presented in a separate paper²⁴). After $S_{660}P$, another phase (denoted by an open circle) is also notable (except for the event 22 July 1990). Two characteristics of this phase are observed: (1) this phase arrives ~35 s after the P wave for a source depth of 518 km, and the relative arrival time decreases to ~25 s for a source depth of about 600 km; (2) the slowness of this phase, shown in Fig. 4b, is determined to be slightly less than that of $S_{660}P$. Detailed observed travel-time and slowness values are listed in Table 1.

On the basis of these observations, this phase is identified using the iasp91 model as an S-P converted wave with a conversion depth of ~920 km ($S_{920}P$). In other words, the existence of a discontinuity near 920-km depth beneath the Tonga subduction zone is strongly suggested. The observation that a

straight line fits through the $S_{920}P$ arrivals in the depth section (Fig. 4a) indicates that this discontinuity is relatively flat in comparison with the 660-km discontinuity. The detailed conversion depths for each event are also shown in Table 1. We also note that several other large amplitude peaks, which may correspond to other phases, are observed in Fig. 4, but none of them lines up as nicely as $S_{920}P$. We may need to invoke a three-dimensional structure, such as the subducting slab, to explain some of those peaks (we see some evidence for $p_{520}P$ ²¹, but not for $p_{210}P$ ¹⁸).

Relative amplitudes of $S_{920}P$ to $S_{660}P$ have a range of 0.25–0.95, with an average near 0.5 (Table 1). According to the iasp91 model, for a focal depth of about 580 km, a reflection of an upgoing p wave at the 410-km discontinuity, $p_{410}P$ is characterized by about the same travel time as $S_{920}P$. Consequently, the overlap of these two waves may produce a large amplitude ratio for events 16 April 1993, 30 August 1992 and 30 September 1991. Omitting these events in the estimation of $S_{920}P/S_{660}P$, the ratio may be reduced to ~0.4. Considering that $S_{920}P$ and $S_{660}P$ are characterized by only slightly different take-off angles during the initial radiation as S waves from the source, similar amplitudes are expected for these two phases before P wave conversion. It is therefore most likely that the S-wave velocity contrast (the amplitude of the S_dP phases depends almost entirely on S-velocity contrast) at the 920-km discontinuity is about 40% of that of the 660-km discontinuity. Theoretical ratios of $S_{660}P/P$ and $S_{920}P/S_{660}P$ are also given in Table 1. They are estimated from synthetic seismograms stacked in the same way as for the observed data. Reflectivity synthetic seismograms²⁵ are calculated assuming known focal mechanisms²⁶ for a modified iasp91 model, which has an extra discontinuity at a depth of 920 km with a velocity jump of 40 percent of the 660-km discontinuity (that is ~2.4% S-wave velocity jump). On average, theoretical and observed $S_{920}P/S_{660}P$ are quite similar, supporting the estimated size of the 920 km discontinuity. However, we could not identify $S_{920}P$ for the 22 July 1990 event (Fig. 4a, b), although the theoretical calculation suggests that $S_{920}P$ should be large enough to be observed. The large discrepancy between observed and theoretical $S_{660}P/P$ suggests that the assumed focal mechanism may be incorrect. The expected arrival times of $S_{920}P$ and $s_{410}P$ are almost the same and the destructive overlap of two phases may be masking their presence in the stacked traces, because only a limited portion of J-array data are available for this event, reducing the slowness resolution in stacking. As to the thickness of the 920-km discontinuity, considering that $S_{920}P$ is also observable in the period range as short as 1–2 s, the thickness of the 920-km discontinuity may be of the order of 10 km.

To see whether the inferred 920-km discontinuity is simply a local feature, we analysed deep earthquakes that occurred in Japan sea (19 January 1993) and in Flores sea (7 June 1991) recorded respectively by the Southern California Seismographic Network and the J-array. Figure 5 shows the stacked results for these two events. Following the direct P arrivals, two notable

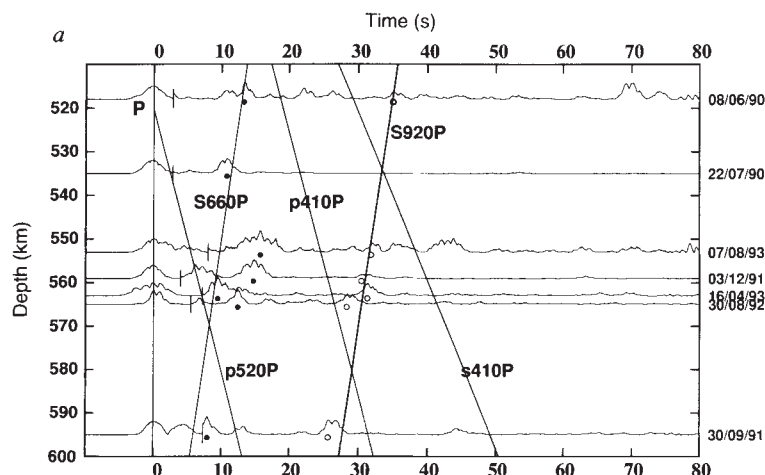


FIG. 4 *a*, Depth section of the stacked traces for a slowness of -0.1 s deg^{-1} . To show later arriving phases more clearly, traces to the right of the vertical line are enlarged so that the amplitude of $S_{660}P$ becomes the same as that of direct P . Theoretical travel times of the iasp91 model for major expected bodywave arrivals at the epicentral distance $\Delta = 70^\circ$ are indicated by straight lines. The thick line indicates the theoretical arrival time of $S_{920}P$. Event dates are given in the order day/month/year. *b*, Colour contour maps of the individual events. Colour scaling is similar to Fig. 3*b*, but modified slightly for each event to help observing major arrivals. Expected arrival times of $S_{920}P$ are indicated by vertical red arrows. White arrows indicate $S_{920}P$.

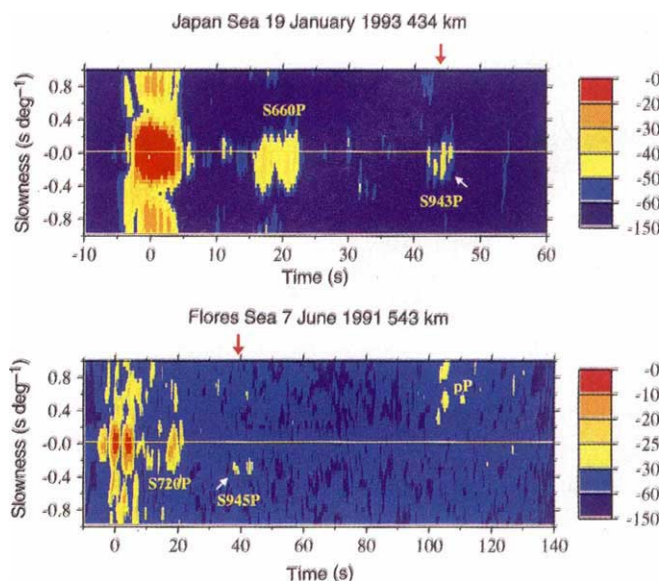
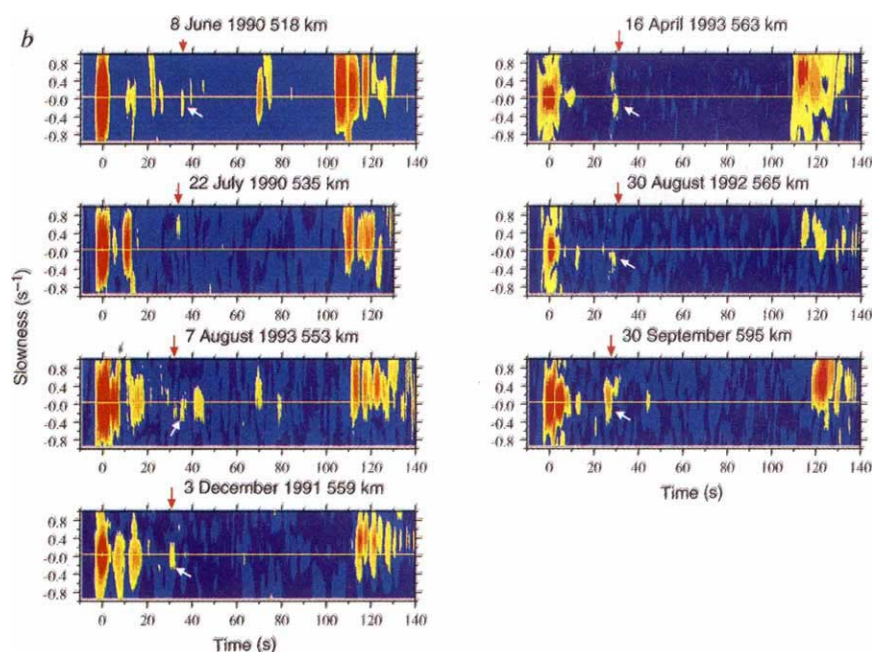


FIG. 5 Stacked section of two events in other subduction zones. The red arrows indicate expected arrival times of $S_{945(3)}P$ as in Fig. 4*b*. For the Flores sea event, the expected slowness of $S_{945}P$ is -0.27 s deg^{-1} . This is smaller than that for the other events because the epicentral distance of the J-array, $\Delta = 45^\circ$, is smaller for this event. The '920-km discontinuity' is also present in these regions.

phases with small negative slowness are evident. Their slowness indicates that these two phases are S-P converted waves. The second phases correspond to conversion depths of 943 km and 945 km for the Japan and Flores seas respectively.

Global feature?

We have shown above the evidence for a 920-km discontinuity in the deep mantle beneath three subduction zones. It is natural to wonder whether or not it is a global feature. Global confirmation of the 920-km discontinuity is, however, limited by the geographical distribution of the occurrence of deep earthquakes and seismic arrays. Consequently, it is difficult to determine whether this discontinuity exists globally or is restricted to the vicinity of subducting slabs.

A straightforward conclusion which may be drawn from present work is that there is a seismic discontinuity with an S-wave velocity jump of a few per cent at a depth around 920 km beneath subduction zones. If such a discontinuity cannot be observed beneath different tectonic regions other than subduction zones, the most likely explanation for the discontinuity is that it represents the bottom of the subducted slab's garnet layer²⁷. Considering that the depths are almost constant beneath three subduction zones, it is unlikely that the layer is dynamically supported as depicted by Ringwood and Irifune²⁷; instead, it should be fairly flat and the depth is thermodynamically controlled. If a stability condition of materials that constitute such a layer is experimentally determined, we should be able to constrain the temperature of the mantle at a depth of ~920 km, which we have no good way to estimate otherwise.

On the other hand, we must also pay attention to the fact that suggestion of either first-order or second-order discontinuities at these depths is not completely new. Earlier this century, Gutenberg noted a second-order discontinuity (a discontinuous velocity gradient) at a depth of 950 km, which defines the bottom of the transition zone²⁸. As mentioned earlier, Bullen also viewed this depth as the base of the transition zone¹. Changes of slowness of P waves bottoming at these depths have been reported by many researchers^{11,29-31}. Reflected phases from this general depth range have also been suggested previously beneath different tectonic regions: subduction zones¹³⁻¹⁵, oceanic ridges³² and continents⁹, although the evidence for the presence of a discontinuity is much weaker than that presented by this study. Recently, analysing precursors to the SS phase, Petersen *et al.*¹³ showed good evidence for a reflector at a depth of 900 km beneath the Kuril-Kamchatka subduction zone. Although the authors do not mention it, the recent work by Gurrola *et al.* (in their Fig. 14a)³³ also suggests a presence of such discontinuity beneath western Russia.

While the question is by no means settled, we thus favour the possibility that the 920-km discontinuity may be a global feature, and that it may represent the bottom of the transition zone, below which the lower mantle should be defined. The geophysical consensus on the depth of the top of the lower mantle has shallowed considerably from ~950 km to ~660 km in the past 30 years⁶. This was probably so because the 660-km discontinuity has attracted so much attention on the part of geophysical community. The confirmation of the existence of the 920-km discontinuity may deepen the depth of the top of the lower mantle back to its original placement by Bullen and Gutenberg. We may also need to introduce primed symbols for Bullen's region C, as Bullen himself noted¹.

The consequence of the presence of a 920-km discontinuity, if its global nature is ultimately confirmed, would be of great importance for many problems in geodynamics. With currently accepted mineralogical models for the upper mantle, if we assume that upper and lower mantles are chemically homogeneous, no known phase transformation exists below a depth of 720 km (ref. 34). It has been suggested that the change in crystal symmetry of perovskite might account for the existence of a discontinuity at about ~900 km (refs 15, 35, 36), but the recent experimental and theoretical studies seem to indicate that orthorhombic perovskite is stable in most of the lower mantle^{37,38}. Funamori and Yagi³⁷ showed that orthorhombic perovskite is stable up to 1,900 K at 36 GPa. This condition is close to that expected for a depth of 920 km with a 'cold geotherm' which can be obtained assuming the whole mantle is convecting as a unit³⁹. It may still be possible that orthorhombic perovskite transforms into a higher symmetry phase at a higher temperature with a 'hot geotherm' to account for the presence of the 920-km discontinuity. Even in this case, a presence of a thermal boundary layer at this depth range would be required, leading to the idea of a two-layered convecting mantle³⁹. If the possibility of the change in crystal symmetry of perovskite is completely ruled out from experimental work, we may need to invoke the idea that the lower mantle is chemically distinct from the upper mantle (that is, the 920-km discontinuity is a chemical boundary, while the 660-km discontinuity is a phase boundary), even though some slab may penetrate through the boundary^{40,41}. So in any case, the implication of the presence of a global 920-km discontinuity would be enormous for our understanding of the mantle dynamics, and further detailed seismological and mineralogical studies of the lower part of the mantle transition zone below the 660-km discontinuity may help to answer the long-standing unsolved problem of Earth sciences: does the Earth's mantle convect in one layer or two? □

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Australopithecus ramidus, a new species of early hominid from Aramis, Ethiopia

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Seventeen hominoid fossils recovered from Pliocene strata at Aramis, Middle Awash, Ethiopia make up a series comprising dental, cranial and postcranial specimens dated to around 4.4 million years ago. When compared with *Australopithecus afarensis* and with modern and fossil apes the Aramis fossil hominids are recognized as a new species of *Australopithecus*—*A. ramidus* sp. nov. The antiquity and primitive morphology of *A. ramidus* suggests that it represents a long-sought potential root species for the Hominidae.

WORK in southern Africa established *Australopithecus* as a human ancestor and revealed specific diversity within that genus. Subsequent work in eastern Africa extended the known geographical and temporal distribution of the genus. Until now, the earliest hominid species known was *Australopithecus afarensis*, dated to between 3 and 4 Myr. *A. afarensis* narrowed the temporal and morphological gap between Miocene hominoids and other early hominids¹. Its primitive craniodental anatomy offered some support for molecular work² which had suggested a late Miocene or early Pliocene age for the common ancestor of hominids and African apes. Because details of the ape and human divergence are poorly understood^{3–9}, taxonomically diagnostic hominoid fossil evidence antedating the existing record of *A. afarensis* has been eagerly anticipated.

Description of *A. ramidus*

Order Primates Linnaeus 1758
Suborder Anthropoidea Mivart 1864
Superfamily Hominoidea Gray 1825

Australopithecus DART 1925

Australopithecus ramidus sp. nov.

Etymology. In recognition of the Afar people who occupy the Middle Awash study area and contribute to fieldwork there. The name is from the Afar language. 'Ramid' means 'root' and it applies to both people and plants.

Holotype. ARA-VP-6/1 (Fig. 1a) is an associated set of teeth from one individual that includes: upper left I¹, C, P³, P⁴, right I¹, C (broken), P⁴, M²; and lower right P₃ and P₄. Found by Gada Hamed on Wednesday, 29 December 1993. Holotype and paratype series housed at the National Museum of Ethiopia, Addis Ababa.

Paratypes. Table 1 lists the holotype and paratype series, all from Aramis. Included are associated postcranial elements, two partial cranial bases, a child's mandible, associated and isolated teeth.

Locality. Aramis localities 1–7 are in the headwaters of the Aramis and Adgantoli drainages, west of the Awash river in the Middle Awash palaeoanthropological study area, Afar depression, Ethiopia¹⁰. Aramis VP Locality 6 is at 10° 28.74' north latitude; 40° 26.26' east longitude; ~625 m elevation.

Horizon and associations. All hominid specimens were surface finds located in the section within 3 m of the Daam Aatu Basaltic Tuff. The immediately underlying Gāala Vitric Tuff Complex is dated at 4.39 ± 0.03 Myr (ref. 10).

Diagnosis. *A. ramidus* is a species of *Australopithecus* distinguished from other hominid species, including *A. afarensis*, by the following: upper and lower canines larger relative to the postcanine teeth; lower first deciduous molar narrow and obliquely elongate, with large protoconid, small and distally placed metaconid, no anterior fovea, and small, low talonid with minimal cuspule development; temporomandibular joint without definable articular eminence; absolutely and relatively thinner canine and molar enamel; lower third premolar more strongly asymmetrical, with dominant, tall buccal cusp and steep, posterolingually directed transverse crest; upper third premolar more strongly asymmetric, with relatively larger, taller, more dominant buccal cusp.

A. ramidus is distinguished as a hominid from modern great apes and known elements of *Sivapithecus*, *Kenyapithecus*, *Orrorin*, *Lufengpithecus* and *Dryopithecus* by the following: canine morphology more incisiform, crowns less projecting, with relatively higher crown shoulders; cupped distal wear pattern on lower canine; mandibular P₃ with weaker mesiobuccal projection of the crown base and without functional honing facet; modally relatively smaller mandibular P₃; modally relatively broader lower molars; foramen magnum anteriorly placed relative to carotid foramen; hypoglossal canal anteriorly placed relative to internal auditory meatus; carotid foramen placed posteromedial to tympanic angle.

A. ramidus is further distinguished from both *Pan* and *Gorilla* by the following: upper canine not mesiodistally elongate.

A. ramidus is further distinguished from *Pan troglodytes* and *Pan paniscus* by the following: upper central incisors small relative to postcanine teeth; lower third molars elongate and larger relative to other molars; molars not as crenulated, occlusal foveae not as broad.

A. ramidus is further distinguished from *Gorilla* by the following: smaller absolute tooth and upper limb size; flatter temporomandibular joint; lack of strong molar cusp relief; less sectorial first deciduous molar, dm₁.

Dental description

The ARA-VP-1/129 child's mandible retains a first deciduous molar (dm₁). The dm₁ has been crucially important in studies of *Australopithecus* since the discovery of the genus 70 years ago, and has been used frequently as a key character for sorting apes and hominids^{11–13}. The Aramis dm₁ is morphologically far closer to that of a chimpanzee than to any known hominid (Fig. 2). It is very small—more than 4 s.d. units below the combined *A. afarensis*/*afarensis* mean. It is at the low end of the common chimpanzee size range ($n=29$) and comparable to the bonobo mean ($n=21$) (Table 2). The apelike Aramis dm₁ lacks the

FIG. 1 a, Holotype specimen, ARA-VP-6/1 upper and lower dentition from a single individual; b, partial adult basicranium, ARA-VP-1/500; c, associated adult arm elements, ARA-VP-7/2. All alignments approximate. See text for descriptions.

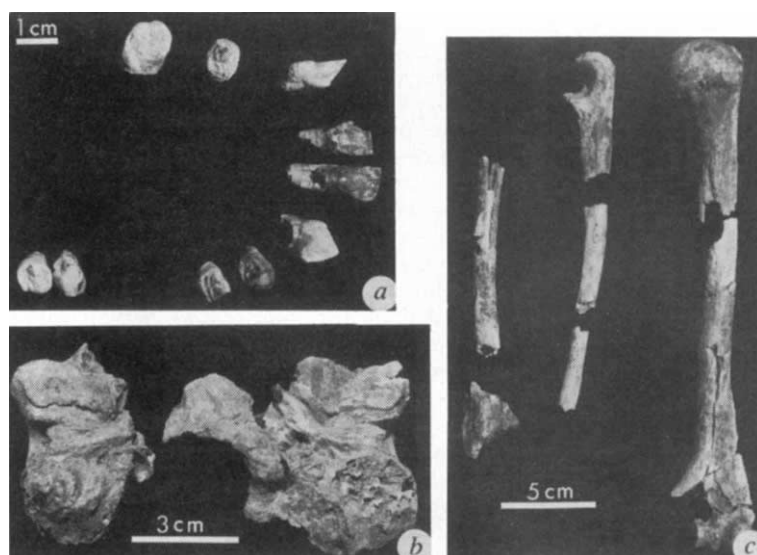
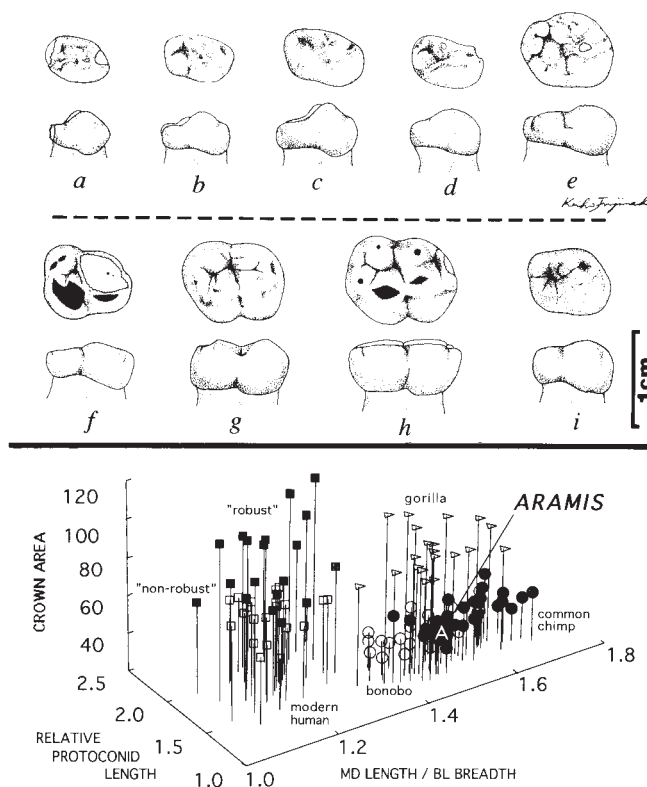


FIG. 2 Deciduous first molar comparisons. Metric and morphological comparisons show a wide separation between the dm_1 of Aramis and those of other early hominid species. a, *Dryopithecus* (IPS 42/1784); b, *Pan paniscus* (T-26992); c, *Pan troglodytes* (PRI 1372); d, *Australopithecus ramidus* (ARA-VP-1/129); e, *A. afarensis* (A.L. 333-43B); f, *A. africanus* (Taung); g, *A. robustus* (TM 1601); h, *A. boisei* (KNM ER-1477); i, *Homo sapiens* (modern). The three-dimensional plot shows dm_1 crown area (buccolingual (BL) multiplied by mesiodistal (MD)) in square mm on the vertical axis. MD length divided by total protoconid length is shown on the left depth axis. The third axis represents a measure of tooth crown narrowness, the MD length divided by the BL breadth. Individual specimens are shown. The 'robust' sample includes *A. robustus*, *A. aethiopicus* (L704) and *A. boisei*. The 'non-robust' sample includes *A. africanus*, *A. afarensis* and early *Homo* (KNM ER-1507; Omo 222). The new species *A. ramidus* is centred in the chimpanzee ranges for these measures. It represents a good ancestral morphotype for all later hominid species.



apparently derived hominid features of buccolingual crown expansion, mesiolingually prominent metaconid, well-defined anterior fovea, and large talonid with well differentiated cusps. The only probable hominid derived feature shared with *A. afarensis* is an occlusally and mesiobuccally reduced protoconid, possibly associated with a loss of deciduous canine honing. The relative size of the talonid, whether judged by relative protoconid

length or actual area ratios, lies at the chimpanzee means. The Aramis tooth stands farther in this feature from *A. afarensis* than *A. afarensis* is separated from robust *Australopithecus* homologues. The crown length to breadth ratio (1.49) shows a very narrow dm_1 , surpassed in mean values only by the common chimpanzee (mean = 1.58) among fossil hominids and modern hominoids. The ratio between labiolingual breadth of the deci-

duous canine root and the square root of the computed dm_1 area shows a relatively large Aramis canine, nearly matching the *Pan paniscus* ratio mean and exceeding the *P. troglodytes* average and the *G. gorilla* range ($n=20$). The only measurable *A. afarensis* specimen (L.H.-2) lies closer to the most extreme *A. boisei* condition (KNM-ER 1477) than it does to Aramis in this ratio.

The *A. ramidus* permanent dentition is represented at most positions (Fig. 3; Table 3). Upper and lower incisors do not exhibit the large size typical of extant *Pan*. Upper and lower central incisor size relative to postcanine teeth is comparable to Miocene hominoids and gorillas. Of the five individuals for whom canine size is determinable, all five have crowns at or larger than the *A. afarensis* mean. Upper and lower canines are also large relative to postcanine teeth. ARA-VP-1/128 is over 5 s.d. units above the *A. afarensis* mean in measures of relative canine size within known individuals (C/P_4 ; C/M_1 ; and C/M_3 ratios of maximum labiolingual canine crown breadth÷square root of computed molar or premolar crown area). In ARA-VP-6/1 relative canine crown area is comparable to the female great ape condition. Morphology of the known Aramis canines, however, diverges from that of known apes (Fig. 3). The upper canines are slightly less incisiform than homologues of *A. afarensis* but more incisiform than any ape counterpart, with occlusally placed terminations of the mesial and distal apical crests (Fig. 3g). The visual result of apically placed crown shoulders is a low, blunt canine tooth relative to more projecting ape canines, a morphological condition which may have important evolutionary implications. The Aramis upper canine is large buccolingually, forming a further contrast with mesiodistally elongate African ape canines. Wear pattern also differs significantly from the ape condition. Mandibular canine wear does not show the pattern typical of great apes. Some worn female *Pan* canines are superficially similar, but still lack the distal crown cupping seen

on Aramis. Instead, they feature planar wear surfaces from contact with the upper canine, even on individuals with rounding (not honing) of the buccal P_3 face.

The broken canines and lower P_3 in ARA-VP-1/128 and -6/1 exhibit thin enamel distinct from previously known hominid conditions. Canine enamel thickness approximates the chimpanzee condition, with a lack of apical thickening we observe in other hominids. The 1.0 mm buccal enamel thickness of the ARA-VP-6/1 broken upper right canine slightly exceeds the 0.9 mm maximum recorded in our small sample of broken female *P. troglodytes* upper canines ($n=6$) and is approximately 2.4 s.d. units above our combined-sex chimpanzee mean of 0.65 mm ($n=14$). The broadly constant enamel thickness of the Aramis maxillary canine above the midcrown height level contrasts with the *A. afarensis* condition in which buccal enamel thickens towards the apex, commonly reaching ~1.5 mm. The significance of maxillary canine enamel thickness can be evaluated in the light of proposed wear mechanics of the C/P_3 complex¹⁴. The relatively thin enamel and large size of the Aramis canine, together with its primitive P_3 morphology, suggest a C/P_3 complex morphologically and functionally only slightly removed from the presumed ancestral ape condition.

The ARA-VP-6/1 P_3 is markedly more apelike than any *A. afarensis* homologue in its high protoconid with extensive buccal face and steep, distolingually directed transverse crest (Fig. 3g). In these features it is indistinguishable from ape homologues. The strong mesiobuccal protrusion of its crown base is also outside the known *A. afarensis* range. The Aramis P_3 deviates toward the *A. afarensis* condition in some details. These include a more occlusal termination of the mesial protoconid crest, weaker mesiobuccal protrusion of the crown base (especially ARA-VP-1/128), and a smaller size relative to P_4-M_3 although rare individual *Pan* specimens do approximate the Aramis condition in

TABLE 1 Aramis fossil hominid specimens

Specimen number	Collection year	Element	Discoverer	Dental dimensions
ARA-VP-1/1	1992	RM ³	G. Suwa	RM ³ : 10.2MD, 12.3BL
ARA-VP-1/2	1992	RI ¹	A. Asfaw	RI ¹ : 8.2LL
ARA-VP-1/3	1992	L _c frag.	G. Suwa	
ARA-VP-1/4	1992	Right humerus, full shaft	S. Simpson	
ARA-VP-1/125	1992	Left temporal	S. Simpson	
ARA-VP-1/127	1992	L ^C , RM ¹ , worn roots of incisors, canine and premolar	T. White	
ARA-VP-1/128	1992	Associated teeth	T. Assebework	L _c : 11.0LL; RP ₃ : 7.5Mn, (9.8)Mx; LP ₃ : 7.5 Mn, (9.9)Mx; RP ₄ : 7.3 (7.5)MD, 9.5BL; LP ₄ : 7.3 (7.5)MD; RM ₁ : 10.9 (11.2)MD, (10.3)BL; LM ₁ : 10.6 (11.0)MD, (10.1)BL; LM ₂ : 12.8 (13.0)MD, 11.9BL; RM ₂ : 12.7(MD), 11.0(BL) RI ₁ : 6.0MD; Rdm ₁ : 7.3MD, 4.9BL
ARA-VP-1/129	1992	Right mandible (I ₁ , dm ₁)	A. Asfaw	
ARA-VP-1/182	1992	RM3 fragment	Group	
ARA-VP-1/183	1992	UC fragment	Group	
ARA-VP-1/200	1993	LM ₁	A. Ademassu	LM ₁ : 11.0MD, 10.3BL
ARA-VP-1/300	1993	R ^C	Y. Haile-Selassie	R ^C : (11.2)MD, 11.1LL, 14.3CH
ARA-VP-1/400	1993	LM ²	Y. Beyene	LM ² : (11.3–12.3)MD, (15.0)BL
ARA-VP-1/401	1993	LM ₃ fragment	M. Feseha	
ARA-VP-1/500	1993	R. + L. temp. + occ.	T. White	
ARA-VP-6/1	1993	Associated teeth	G. Hamed	LI ¹ : 9.6 (10.0)MD, 7.5LL, 12.5swCH; R ^C : 11.6LL, 14.5CH; L ^C : 11.7LL, 14.6CH, 11.5MD; LP ³ : 7.7MD, 8.4MxMD; 12.5BL; RP ⁴ : 8.4MD, (11.3)BL; RM ² : 11.8MD, (14.1)BL; RP ₃ : (8.2)MnMD; (11.5)MxBL; RP ₄ : 8.9MD, 9.7BL
ARA-VP-7/2	1993	Left humerus, radius, ulna	A. Asfaw	

Fossil hominid specimens recovered from Aramis between December 1992 and December 1993. Dental dimensions are standard, estimates for breakage or interproximal attrition are shown in parentheses. BL, Buccolingual; LL, labiolingual; MD, mesiodistal; CH, distance from buccal enamel line to apex (canine height); Mn, minimum diameter; Mx, maximum diameter; sw, slightly worn. All measurements were taken on original specimens by the authors.

these features. The worn ARA-VP-1/128 P_3 lacks a honing facet but exhibits steep mesial and distal wear slopes not matched in *A. afarensis*.

The P^3 is distinctly primitive in its tall and mesiodistally elongate paracone. Both P^3 and P^4 exhibit a prominent anterior transverse crest. In the P^3 this crest defines an anteriorly facing triangular portion of the occlusal surface, as in apes. The lower P_4 exhibits a prominent transverse crest and minimal talonid development. The P_4 s from two known individuals are both single rooted.

Molar morphology resembles the *A. afarensis* condition, but lacks the extreme buccolingual breadth relative to mesiodistal length common in that species (Fig. 3a–e). The 'serrate' root pattern and deep dentine wear on the buccal cusps described in *A. afarensis*, Tabarin, and Lothagam^{15–17} also occur in Aramis

specimens. All molars lack the extensive crenulation and broad occlusal foveae characteristic of modern chimpanzees, or the high cusp topography of gorillas. The Aramis lower third molar is rounded distally, like *A. afarensis* and Miocene hominoids. A great size discrepancy between M_1 and M_2 is seen in ARA-VP-1/128.

A distinct difference from known hominids occurs in molar enamel thickness. Maximum radial enamel thickness of crown faces can be measured in three fractured Aramis specimens and it ranges from 1.1 to 1.2 mm buccally, at or near the unworn cusp apex, perpendicular to the enamel–dentine junction. These values are comparable to the uppermost range of our homologous enamel thickness values measured on broken *P. troglodytes* molars ($n=22$; M_1 through to M_3). Equivalent measures in *A. afarensis* range from 1.4 to 2.0 mm ($n=5$). In one case (the

TABLE 2 Lower first deciduous molar (dm_1) measurements

	Mesiodistal (MD) length	Buccolingual (BL) breadth	MD × BL area	Protoconid length	MD Length + protoconid length
Aramis ($n=1$)	7.3	4.9	35.8	5.2	1.4
<i>A. afarensis</i>					
<i>n</i>	4	4	4	4	4
min	8.5	7.6	68.0	4.3	1.7
max	9.6	8.4	80.6	5.6	2.0
mean	9.2	7.9	72.5	5.1	1.8
s.d.	0.5	0.4	5.7	0.6	0.1
<i>A. africanus</i>					
<i>n</i>	7	5	5	3	3
min	8.4	7.1	59.6	5.2	1.6
max	9.1	8.1	73.7	5.3	1.7
mean	8.8	7.6	66.6	5.2	1.7
s.d.	0.2	0.4	5.5	0.1	0.1
<i>A. robustus</i>					
<i>n</i>	8	8	8	8	8
min	9.0	7.7	71.0	4.3	1.8
max	10.8	9.7	101.9	5.8	2.3
mean	10.1	8.3	83.7	4.9	2.1
s.d.	0.5	0.6	9.5	0.5	0.1
<i>P. paniscus</i>					
<i>n</i>	21	21	21	20	20
min	6.3	4.4	27.7	4.3	1.3
max	8.8	5.5	48.4	6.0	1.6
mean	7.4	5.1	37.6	5.0	1.5
s.d.	0.6	0.31	4.7	0.5	0.1
<i>P. troglodytes</i>					
<i>n</i>	29	29	29	29	29
min	7.0	4.6	32.9	5.0	1.3
max	9.4	5.8	54.5	6.7	1.6
mean	8.1	5.2	42.2	5.8	1.4
s.d.	0.6	0.4	5.2	0.5	0.1
<i>G. gorilla</i>					
<i>n</i>	20	20	20	20	20
min	9.8	6.7	71.4	6.7	1.3
max	12.2	8.9	108.6	9.0	1.6
mean	11.0	7.5	82.3	7.8	1.4
s.d.	0.7	0.6	10.7	0.6	0.1
<i>P. pygmaeus</i>					
<i>n</i>	6	6	6	6	6
min	8.4	6.4	53.8	5.8	1.3
max	10.2	8.1	82.6	8.1	1.5
mean	9.2	7.1	66.2	6.7	1.4
s.d.	0.7	0.6	10.3	0.8	0.1
<i>H. sapiens</i>					
<i>n</i>	21	21	21	21	21
min	7.4	6.4	47.4	4.0	1.4
max	9.2	8.1	69.9	5.7	2.1
mean	8.4	7.2	60.4	4.9	1.7
s.d.	0.5	0.4	6.1	0.5	0.2

Comparative metrics on deciduous lower first molars (dm_1) of various hominoid taxa. Abbreviations and conventions as in Table 1.

ARA-VP-1/128 third molar) Aramis radial enamel thickness at the buccal protoconid face can be evaluated relative to cervical breadth. A comparison of this ratio of enamel thickness suggests that *A. ramidus* may be characterized as intermediate between the chimpanzee and the *A. afarensis*/*affricanus*/early *Homo* conditions.

In postcanine size, the range of the available Aramis sample includes specimens smaller than known *A. afarensis* homologues (the two known M_1 teeth are both more than 3 s.d. units below the mean). Of the seven Aramis individuals for whom postcanine tooth size is determinable, all have crown sizes smaller than the *A. afarensis* mean. We interpret the limited morphology and metrical data available as indicating a single species with a postcanine dentition significantly smaller than in *A. afarensis*.

The postcanine mandibular row can be reconstructed for ARA-VP-1/128 by juxtaposing interproximal facets (Fig. 3a–c). This shows that the C to M_2 dental row is weakly concave buccally, as in modern and fossil apes and some *A. afarensis* speci-

mens. The P_3 axis is less oblique than in most apes. The canine is positioned directly in line with the mesiodistal axis of the postcanine tooth row rather than being set mesiolingually to the postcanine axis as in the case for most *A. afarensis*. This is a more primitive arrangement shared with modern and Miocene great apes, and may suggest that the mesial part of the lower canine was not functionally incorporated into the incisal row as seen in *A. afarensis*¹⁷.

Cranial description

The ARA-VP-1/125 and -1/500 specimens represent adult temporal and occipital regions (Fig. 1b). Both are smaller than their *A. afarensis* counterparts, but no female temporal is known for that species. The Aramis cranial fossils evince a strikingly chimpanzee-like morphology that includes marked pneumatization of the temporal squama which even invades the root of the zygoma. The occipital condyle is small, measuring 16×7.5 mm. The anterior border of the foramen magnum (basion) is intersected by a bicarotid chord connecting the centres of right and

TABLE 3 Comparative dental metrics for permanent dentition

a, Upper dentition																		
		<i>n</i>	Min	Mesiodistal				<i>n</i>	Min	Labio/buccolingual				<i>n</i>	Crown area (MD × BL)			s.d.
				Max	Mean	s.d.			Max	Mean	s.d.			Min	Max	Mean		
I ¹																		
	<i>A. afarensis</i>	3	10.8	11.8	11.2	0.6	5	7.1	8.6	8.20	0.6	3	90.5	99.1	94.2	4.5		
	Aramis	1	—	—	(10.0)	—	2	7.5	8.2	—	—	1	—	—	(75.0)	—		
C																		
	<i>A. afarensis</i>	9	8.9	11.6	10.0	0.8	10	9.3	12.5	10.9	1.1	9	82.8	145.0	109.9	18.9		
	Aramis	2	(11.2)	11.5	—	—	2	11.1	11.7	—	—	2	(124.3)	134.5	—	—		
P ³																		
	<i>A. afarensis</i>	9	7.5	9.3	8.7	0.5	8	11.3	13.4	12.4	0.6	8	84.7	120.9	108.0	11.0		
	Aramis	1	—	—	7.7	—	1	—	—	12.5	—	1	—	—	96.3	—		
P ⁴																		
	<i>A. afarensis</i>	10	7.6	9.7	9.0	0.6	6	11.1	12.6	12.1	0.6	6	84.4	119.7	106.8	12.6		
	Aramis	1	—	—	8.4	—	1	—	—	(11.3)	—	1	—	—	(94.9)	—		
M ²																		
	<i>A. afarensis</i>	5	12.1	13.5	12.8	0.5	6	13.4	15.1	14.7	0.6	5	162.1	199.8	187.5	14.6		
	Aramis	2	(11.8)	11.8	—	—	2	(14.1)	(15.0)	—	—	2	(166.4)	(177.0)	—	—		
M ³																		
	<i>A. afarensis</i>	8	10.5	14.3	11.9	1.4	8	13.0	15.5	13.8	1.0	8	136.5	215.9	165.1	30.9		
	Aramis	1	—	—	10.2	—	1	—	—	12.3	—	1	—	—	125.5	—		
b, Lower dentition																		
		<i>n</i>	Min	Mesiodistal				<i>n</i>	Min	Labio/buccolingual				<i>n</i>	Crown area (MD × BL)			s.d.
				Max	Mean	s.d.			Max	Mean	s.d.			Min	Max	Mean		
I ₁																		
	<i>A. afarensis</i>	2	6.2	8.0	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Aramis	1	—	—	6.0	—	—	—	—	—	—	—	—	—	—	—	—	
C																		
	<i>A. afarensis</i>	—	—	—	—	—	13	8.8	12.4	10.4	1.1	—	—	—	—	—	—	
	Aramis	—	—	—	—	—	1	—	—	11.0	—	—	—	—	—	—	—	
P ₃ (min/max)																		
	<i>A. afarensis</i>	19	6.5	9.8	8.6	1.1	19	9.7	13.3	11.6	1.1	19	63.1	127.7	99.7	20.4		
	Aramis	2	7.5	(8.2)	—	—	2	(9.9)	(11.5)	—	—	2	(74.2)	(94.3)	—	—		
P ₄																		
	<i>A. afarensis</i>	15	7.7	11.1	9.7	1.0	14	9.8	12.8	10.9	0.8	14	77.0	129.7	106.5	16.8		
	Aramis	2	7.5	8.9	—	—	2	9.5	9.7	—	—	2	71.2	86.3	—	—		
M ₁																		
	<i>A. afarensis</i>	17	11.2	14.0	13.0	0.6	16	11.0	13.9	12.6	0.8	16	124.3	194.6	164.9	17.1		
	Aramis	2	11.0	11.1	—	—	2	(10.2)	10.3	—	—	2	(113.2)	113.3	—	—		
M ₂																		
	<i>A. afarensis</i>	23	12.4	16.2	14.3	1.0	22	12.1	15.2	13.5	0.9	22	152.5	234.1	193.3	24.3		
	Aramis	1	—	—	(13.0)	—	1	—	—	11.9	—	1	—	—	(154.7)	—		
M ₃																		
	<i>A. afarensis</i>	14	13.7	16.3	14.8	0.8	14	12.1	14.9	13.3	0.8	13	172.0	231.5	195.7	17.7		
	Aramis	1	—	—	12.7	—	1	—	—	11.0	—	1	—	—	139.7	—		

Comparative metrics for the permanent teeth of *A. afarensis* (comprises the Hadar pre-1990 sample and the full Laetoli and Maka samples) and *A. ramidus* (from Table 1). Data are shown only for tooth positions represented in both species. Measurements are standard and were taken by the authors on original specimens with conventions and abbreviations as in Table 1. There is considerable overlap between the known species ranges, as there is among other species in the genus. As documented in the text and illustrations, however, proportional differences within individual dentitions combine with other morphological considerations to warrant the recognition of *A. ramidus* as a species distinct from *A. afarensis*.

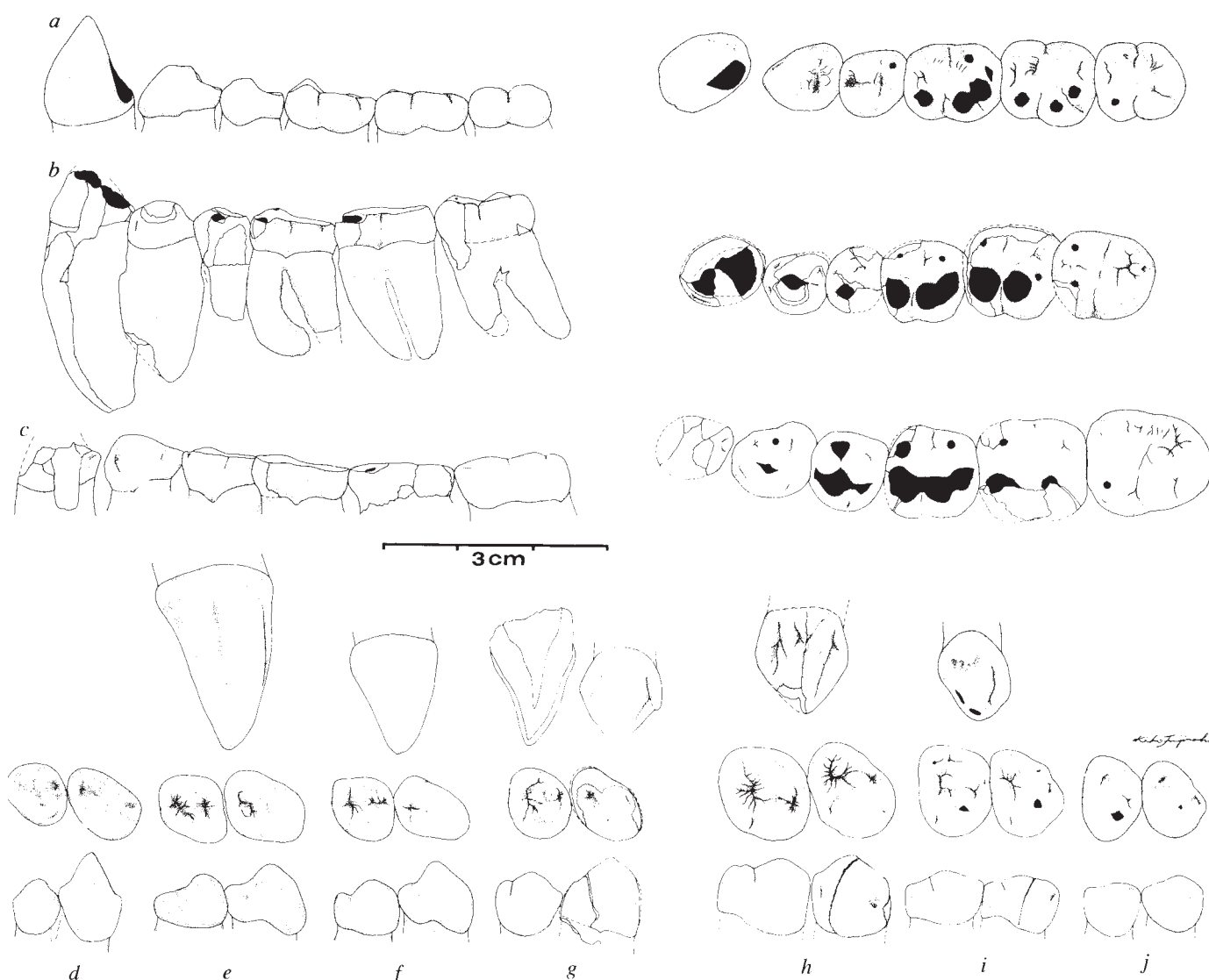


FIG. 3 Comparisons of upper canine/lower premolar complexes and tooth rows. Top three rows, Occlusal and lateral views of the lower tooth rows of: a, *Pan troglodytes* female (CMNH B1770); b, *A. ramidus* (ARA-VP-1/128); c, *A. afarensis* holotype (Laetoli Hominid 4). Bottom three rows, Lingual views of upper canines and occlusal and buccal views of

lower third and fourth premolars of: d, *Dryopithecus* (MNHN); e, *Pan troglodytes* male (CMNH B1882); f, *P. troglodytes* female (CMNH B1721); g, *A. ramidus* holotype (ARA-VP-6/1; split right canine on the left); h, *A. afarensis* (LH-3); i, *A. afarensis* (A.L. 400); j, *A. afarensis* (A.L. 288-1, 'Lucy'). a, c and h reversed for comparison.

carotid foramina, and the endocranial opening of the hypoglossal canal is placed more anteriorly relative to the internal auditory meatus than in great apes. This condition, as in other fossil hominid taxa, reflects a shortened basioccipital component of the cranial base relative to modern African ape crania. The temporomandibular joint is very flat, with virtually no articular eminence and weak inferior projection of the entoglenoid process. The tympanic is tubular, bounded anteriorly and posteriorly by deep furrows, and the tube extends to the lateral edge of the postglenoid process in one individual and beyond it in the second. The mastoid process is a blunt eminence rather than the inflated, inflected pyramidal structure diagnostic of the chimpanzee. The digastric groove is distinctly deeper than in the chimpanzee.

Postcranial description

The ARA-VP-7/2 specimen (Fig. 1c) is a rare association of all three bones from the left arm of a single individual. In size the specimen indicates a hominid larger than the A.L. 288-1 *A. afarensis* from Hadar and smaller than other individuals of this species. Fracture of the specimen currently precludes length esti-

mates for the three elements, but the humeral head is approximately 30% larger than the smallest (A.L. 288-1) *A. afarensis* specimen (breadth: Aramis = 34.6, A.L. 288-1 = 27.0; height: Aramis = 36.5, A.L. 288-1 = 28.1). The arm displays a mosaic of characters usually attributed to hominids and/or great apes. From proximal to distal, probable derived characters shared with other hominids include an elliptical humeral head; a blunt, proximally extended ulnar olecranon process surmounting a straight dorsal upper shaft profile; an anteriorly oriented trochlear notch; and, an anteriorly facing ulnar brachialis insertion. The specimen also shows a host of characters usually associated with modern apes, including a strong angulation of the distal radial articular surface due to a large styloid process, a strong lateral trochlear ridge on the distal humerus (also seen in some *A. afarensis*), and an elongate, superoposteriorly extended lateral humeral epicondyle. The Aramis arm diverges from the African ape condition in other features. The proximal humerus lacks the deep, tunnel-like bicipital groove often seen on African apes. Further studies will unravel the functional and phylogenetic significance (polarities) of these and other postcranial characters.

Comparisons and remarks

The pre-5 Myr record of hominid evolution is sparse. Although the Lothagam fragment has been attributed to *A. cf. afarensis*^{18, 20} this assignment was made mostly on the basis of primitive characters and in the absence of associated cranial, anterior dental or postcranial remains. Hominid remains from the period between 4 and 5 Myr are also few and poor, comprising a proximal humerus and jaw fragment from Baringo^{16, 19, 22}, and a distal humerus from Kanapoi of more uncertain age. These and the slightly younger Kubi Algi^{23, 24} and Fejej²⁵ specimens have all been attributed to *A. afarensis*.

To our knowledge, no fossils predating 4 Myr have been identified as representing taxa other than *Australopithecus afarensis* and *Australopithecus africanus*^{16, 19, 20, 23, 26}. Assignment of the limited available >4 Myr sample to *A. afarensis* was warranted for the comparatively undiagnostic Lothagam, Baringo, Kanapoi and Tabarin specimens from Kenya¹⁶. The discovery of more complete, more diagnostic specimens at Aramis allows a recognition of characters which distinguish them at (minimally) the species level from Hadar, Maka and Laetoli hominid fossils. The limited preserved morphology in the Lothagam, Tabarin and Baringo specimens broadly matches both the Aramis sample and *A. afarensis*. The discovery of the Aramis hominids demonstrates, however, that some of the suggested differences between Lothagam and *A. afarensis* (for example, enamel thickness¹⁵) are likely to be substantiated. However, the preserved anatomy of these Kenyan specimens may well reflect primitive character states for the basal hominid (and perhaps ancestral hominoids). Nothing available for these Kenyan specimens validates inclusion in the new Ethiopian taxon before the recovery of more diagnostic body parts.

We note that Ferguson, referring to casts and literature, has invented a plethora of new names for early African hominids (for instance, he divides *A. afarensis* into three species; an alleged dryopithecine ape²⁷, a diminutive early human²⁸, and a subspecies of *A. africanus*²⁹). His invalid naming of the A.L. 288 specimen as 'Homo antiquus' (in which he includes KNM-ER 1813)²⁸ was followed by his 1989 placement of the Baringo Tabarin specimen (which he incorrectly identified as 'KNM-ER TI 13150') into a subspecies ('praegens') of that species³⁰. Because of these problems, because Ferguson's diagnosis of that specimen did not differentiate it from *A. afarensis*, and because it lacks any characters that differentiate it from the latter species or unequivocally link it to the Aramis species¹⁶, we consider Ferguson's subspecific nomen 'praegens' to be a *nomen dubium* and propose that it be suppressed even in the event that the

Tabarin specimen be shown conspecific with the Aramis series.

The 1992/93 Aramis hominids share a wide array of traits with *A. afarensis* but also depart anatomically from this species in lacking some of the key traits it possesses and which are shared exclusively among all later hominids. Because of relationships indicated by molecular studies, and because terminal Miocene to Pleistocene fossil African apes are unknown, comparison of the Aramis hominids and modern African apes is warranted. The Aramis remains evince significant cranial, dental and postcranial similarities to the chimpanzee condition, but some or all of these features may be primitive retentions. Only further discoveries and comparisons may elucidate which features actually define the chimp-human and/or African ape-human clades. Meanwhile, the modern African apes are distinct in many dental features from both Aramis and middle to late Miocene hominoids, and thus probably do not represent the ancestral condition^{8, 9}. At the same time, the relatively thin Aramis molar enamel suggests that a simple "hard object feeder" model⁷ is likely to be inaccurate for the ancestral African ape/hominid stock.

We have taken a conservative position here regarding placement of the Aramis fossils at the family and genus levels. The major anatomical/behavioural threshold between known great apes and Hominidae is widely recognized to be bipedality and its anatomical correlates. The two derived craniodental characters shared among all hominids are anterior placement of the occipital condyle/foramen magnum and a more incisiform canine with reduced sexual dimorphism. Acquisition of these states at Aramis may correlate with bipedality^{31, 32} although this remains to be demonstrated. The postcranial evidence available for *A. ramidus* is not definitive on the issue of locomotor pattern.

The anticipated recovery at Aramis of additional postcranial remains, particularly those of the lower limb and hip, may result in reassessment of these fossils at the genus and family level. Meanwhile, characters such as the modified C/P3 complex, an anterior foramen magnum, and proximal ulnar morphology (shared with later *Australopithecus* species) suggest that the Aramis fossils belong to the hominid clade. Similarity to the *A. afarensis* hypodigm warrants the inclusion of the Aramis fossils in the genus *Australopithecus*. At the same time, *A. ramidus* is the most apelike hominid ancestor known, and its remains suggest that modern apes are probably derived in many characters relative to the last common ancestor of apes and humans. More work at Aramis should further elucidate the sexual dimorphism, locomotion, diet and habitat of this species. The fossils already available indicate that a long-sought link in the evolutionary chain of species between humans and their African ape ancestors occupied the Horn of Africa during the early Pliocene. □

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Evidence for a quasar in the radio galaxy Cygnus A from observation of broad-line emission

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ACTIVE galaxies are thought to be powered by the accretion of surrounding dust and gas onto central black holes. The unified model^{1–3} of active galaxies predicts that narrow-line radio galaxies (NLRGs), which are very luminous at radio wavelengths and have narrow optical emission lines, would appear as quasars—which have broad emission lines—if viewed from a different direction. The nearby NLRG Cygnus A is the most luminous radio source in the local Universe, with a luminosity that lies within the range of that of quasars⁴, but the polarization of optical light from this source seems at odds with the quasar model. Here we present evidence, in the form of broad ultraviolet line emission from singly ionized magnesium, that Cygnus A does indeed host a quasar.

Baade and Minkowski⁵ definitively identified this prototypical extremely powerful radio source with an optical galaxy 40 years ago. Jennison and Das Gupta⁶ showed that it is a double radio source, the first known. In terms of radio power, it is comparable with the much more distant ($z \geq 1$) radio galaxies that have been discovered in the past few years³⁷.

The famous apparent double optical nucleus in Cygnus A is extremely strange in that the northwest nucleus consists mostly of high-ionization line emission, while the southeast nucleus is made mostly of continuum light.⁷ (This spatial separation is unexpected because the emission-line regions are thought to be photoionized by the continuum⁸.) The optical featureless continuum source (southeast nucleus) has the surprising property that it is spatially resolved with a size of ~ 3 arcsec which is ~ 3 kpc for $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (ref. 9). A black hole should produce energy in a region far too small to be resolved spatially.

Pierce and Stockton⁹ suggested that the southeast nucleus is a scattering zone reflecting the optical light from a hidden quasar, consistent with the unified model. Subsequently, Tadhunter *et al.*¹⁰ obtained an optical polarization image which was somewhat supportive of this idea in that the polarization vectors lie roughly perpendicular to the direction to the true nucleus, as determined by the compact radio source. However, the percentage polarization is low ($\leq 3\%$), too low in our opinion for scattering in a spatially resolved region in which $\geq 20\%$ is expected. (The low observed polarization is apparently in sharp contrast to the luminous NLRGs at high redshift, which do have high observed polarization^{11–14}.) Furthermore, some of the polarization of Cygnus A is present in the narrow lines as well as the continuum¹⁵. There appears to be too little starlight to dilute scattering polarization to the observed value in the optical¹⁶, although the exact starlight fraction is disputed¹⁷. Finally, arguing against the hypothesis that the optical featureless continuum is primarily reflected quasar light are the low upper limits on the fluxes of any broad lines, and on the equivalent widths of any broad lines in either total flux or polarized flux^{15,17–19}.

Figure 1 shows an ultraviolet spectrum of the southeast nucleus of Cygnus A, obtained with the Faint Object Spectrograph on board the Hubble Space Telescope on 23 May 1993. The observations were made with the 4.3×1.4 arcsec aperture oriented with the long axis at a position angle of 134° (southeast–northwest). This samples light from 1×3 arcsec ($\sim 1 \times 3$ kpc)

away from the true (radio) nucleus. We obtained a 5,716-s exposure with the G270H grating and the red detector, which provides a good-quality spectrum in the range 2,230–3,300 Å, with a dispersion of 2.05 Å per diode. Data from the fine guidance sensors indicate a pointing accuracy of ~ 0.003 arcsec.

The most important feature in the ultraviolet spectrum is the broad Mg II emission line with full width at half maximum (FWHM) $\sim 7,500 \text{ km s}^{-1}$ and an equivalent width of ~ 25 Å. Together with the very high nuclear luminosity⁴, the detection of a broad line shows unambiguously that Cygnus A contains a quasar. Thus, we are led to a scenario in which the ultraviolet is dominated by reflected quasar light (broad emission lines and high polarization) while the optical is dominated by *in situ* extended emission (no broad lines and low polarization). The Mg II equivalent width of ~ 25 Å compared to ~ 50 Å in most quasars is consistent with our scenario in which the continuum flux at 2,800 Å is a combination of reflected quasar light in the ultraviolet and an extension of the optical featureless continuum.

Reddening presents a major challenge to interpreting the spectrum. Some evidence, based on the Cygnus A halo colour and the colours of two neighbouring galaxies, suggests that the Galactic reddening is $E(B-V) \approx 0.35\text{--}0.40$ (refs 7, 20, 21). In support of this value, the Galactic H I column is estimated at $3 \times 10^{21} \text{ cm}^{-2}$ (J. Lockman, personal communication). We tentatively adopt a Galactic reddening of $E(B-V) = 0.4$. If we de-redden our spectrum by this amount (assuming Galactic extinction only), our best-fit power-law has the form $F_\nu \propto \nu^{-2}$. In principle, the slope

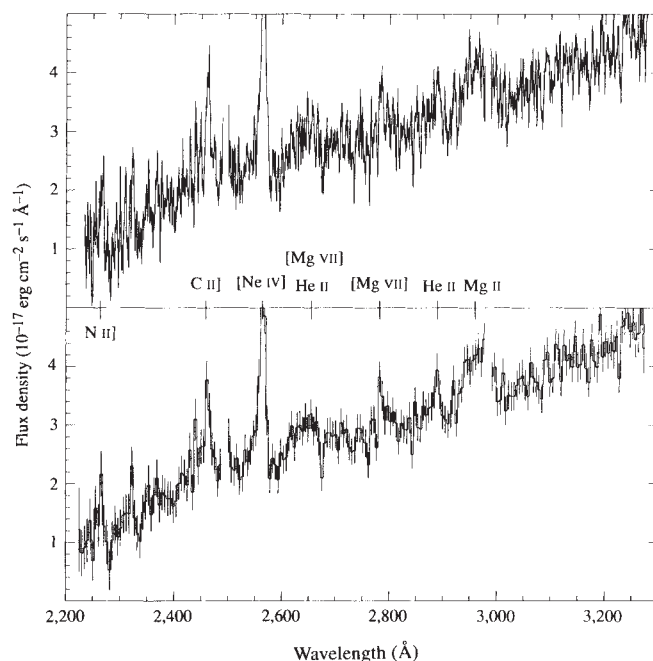


FIG. 1 Top, Calibrated spectrum of Cygnus A, smoothed by one diode which because of substepping corresponds to four pixels on the plots. Bottom, The same spectrum, binned by 5.1 Å per bin with 1σ statistical errors plotted. Two regions in the spectrum had anomalous spikes and have since been identified as noisy diodes. The affected pixels have been removed from the plots. The spectrum is shown at the observed wavelength and no corrections for either Galactic or host reddening have been applied. We have chosen not to present the shorter-wavelength data obtained with the G160L grating. The ends of that spectral region are contaminated by strong geocoronal Ly- α emission in both first and second order from the grating. In addition, the low count rate makes those data susceptible to systematic errors in the background of the Faint Object Spectrograph. Statistical errors dominate the spectrum, and it can be seen from comparison with, for example, the ultraviolet spectrum of NGC1068 that most of the apparently significant features in the Cygnus A spectrum correspond to real features^{23,38}.

provides a clue to the emission and scattering mechanisms, but this value is still affected by reddening in the Cygnus A host. The somewhat unreliable short-wavelength spectrum (1,150–2,220 Å) is higher at $\sim 2,100$ Å than expected for this Galactic reddening, suggesting that some of the reddening might be at the redshift of Cygnus A. This ambiguity does not affect our conclusions.

The broad Mg II emission is probably scattered into the line of sight, as in the nearby Seyfert 2 galaxy NGC1068 (refs 22, 23). In both cases, a naturally occurring cloud in the host galaxy acts as a periscope, enabling us to see the hidden nucleus. The normal broad line ratios in Cygnus A may be affected by a small-particle scattering law. For example, a typical intrinsic ratio of line fluxes $F(H\alpha)/F(\text{Mg II})$ in the broad-line region is 2 (ref. 8) but for Rayleigh scattering, we expect as little as $2(2,800/6,563)^4 = 0.07$. (The Rayleigh scattering example is an extreme limit, closely approximated in two cases of scattered light in active galaxies, the “northeast knot” in NGC1068 (ref. 24) and the off-nuclear dust cloud in PKS2152–69 (ref. 25).) In Cygnus A, the observed broad Mg II flux of $8 \times 10^{-16} \text{ erg cm}^{-2} \text{ s}^{-1}$ corresponds to a broad H α flux of as little as $1.8 \times 10^{-16} \text{ erg cm}^{-2} \text{ s}^{-1}$ with Galactic reddening as estimated above. This is far below the level that would be apparent on any published plot. In fact, the available limits on broad optical line fluxes are sufficiently high that electron scattering from warm ionized gas is also viable.

The polarization of the spatially resolved optical featureless continuum is very low, and the broad lines are not seen in either total flux or polarized flux. Thus, it is unlikely that the extended optical light is predominantly reflected from a hidden quasar. (If the scatterers have a very high velocity dispersion, the emission lines can be washed out, but the appearance of the Mg II line precludes this in Cygnus A, at least in the ultraviolet.) Instead, this extended optical continuum may be the same as the spatially-extended featureless continuum inferred to exist in many narrow-line active galactic nuclei by a spectropolarimetric argument^{26–31}. This extended optical component is slightly polarized, possibly by host interstellar dust transmission, and we believe it dominates the polarized flux in the optical, accounting for the fact that the broad lines have undetectably low equivalent widths in polarized flux as well as in total flux. Recognition of extended optical emission, produced *in situ* rather than reflected, is an important step towards understanding Cygnus A and other active galaxies.

The extended, direct optical component does not have obvious spectral features of young stars. For example, no Balmer edge is seen and probably no $\sim \nu^{-1}$ spectrum in this wavelength range as in the “warmer” models of Terlevich and Melnick³². Perhaps the spectrum could be modelled with reddened OB stars. Very speculatively, we suggest that the spatially extended continuum could also be optically thin thermal emission from highly photo-ionized gas of $10^4 \text{ K} < T < 10^6 \text{ K}$. The intrinsic spectrum in the optical has a shape^{19,33} consistent with free-free emission. In principle, this can be checked with radio observations, although dynamic ranges of published maps of Cygnus A are inadequate for this.

Cygnus A has a low optical polarization whereas other luminous NLRGs have high optical polarizations. This is simply due to the low redshift of Cygnus A (refs 10 and 34). We expected (based on the detection of broad Mg II emission) that Cygnus A would have high polarization in the rest-frame ultraviolet, where the other powerful NLRGs are observed. A preliminary analysis of Hubble Space Telescope ultraviolet imaging of Cygnus A yields an integrated polarization of $6.0\% \pm 1.7\%$ at a position angle of $17^\circ \pm 8^\circ$. This is perpendicular to the radio axis of 285° (ref. 35) and agrees with the “composite model” of ref. 17, at $\lambda \approx 3,200$ Å. (The ultraviolet polarized flux is extended and not simply associated with the southeast nucleus.) Similarly, we expect the distant, luminous NLRGs to show broad ultraviolet emission lines when observed to reasonable continuum sig-

nal-to-noise ratios. Such lines have just been reported in 3C226, 3C277.2 and 3C324 which show broad Mg II emission³⁶; see also the composite spectrum given by McCarthy³⁷ but note that weak broad wings may not appear in the bottom panel of his Fig. 1 because best-fit gaussian emission lines have been subtracted. As in Cygnus A, the equivalent widths in total flux may be somewhat diluted relative to the typical quasar by the direct continuum component. □

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Asteroids falling into the Sun

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THE orbits of comets and near-Earth asteroids evolve chaotically, mainly in response to the gravitational influence of the planets. For comets, it is known that such perturbations can result in trajectories that either collide with or graze the Sun^{1–3}. Indeed, it has been calculated³ that 6% of the known short-period comets (including comet Encke) will become Sun-grazers on a timescale of $\sim 10^5$

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years. But there has been no previous indication that asteroids may suffer a similar fate. To address this question, we have integrated numerically the orbits of several near-Earth asteroids. We find that these asteroids can also undergo solar collisions, through several dynamical routes involving orbital resonances with the giant planets, on timescales of the order of 10^6 years. Of the 47 objects studied, we found 19 cases in which the asteroid collided with the Sun, implying that solar collisions are a more common fate than planetary collisions or ejection from the Solar System. Past Sun-grazing events may also have been recorded in the surface composition and spectral properties of some existing near-Earth asteroids.

In order to understand the dynamics of near-Earth asteroids (NEAs) over timescales comparable to their lifetimes and the evolutionary pathways between different types of orbits, we have integrated numerically the orbits of two samples of NEAs. The first sample consisted of 26 objects, whose secular frequencies of perihelion and nodal longitudes put them close to the main secular resonances, in most cases the ν_6 resonance (ref. 4 and Ch.F. *et al.*, manuscript in preparation; secular resonances occur when the precession rates of the orbits of the asteroid and the planets are commensurable^{5,6}, and ν_6 occurs when the asteroid's perihelion is precessing at the same average rate as that of Saturn). We chose this sample because it has been recently pointed out^{7,8} that the ν_6 resonance provides a very effective mechanism for meteorite transport from the main asteroid belt to the Earth. The second sample consisted of 20 NEAs with osculating orbital elements similar to those of the Taurid meteoroid complex^{9,10} and its likely parent comet Encke (G.B.V. *et al.*, manuscript in preparation); it has been shown³ that this comet becomes a Sun-grazer within a few hundreds of thousand years. All NEAs in our two samples have current semimajor axes a between 1.5 and 3 AU; in the former group, typical eccentricities (e) are lower than or close to 0.6, whereas in the latter group they are higher ($0.6 < e < 0.85$). Table 1 lists the objects belonging to the two groups and considered in our study, and provides information on their fate if they were found to hit the Sun or to be ejected on hyperbolic orbits at some time in the past or the future. As comet Encke has an orbit similar to those in the Taurid group, its orbit has also been integrated and the results included in the Table.

The integrations were carried out with either a Burlisch-Stoer¹¹ or a fifteenth-order Radau¹² variable-stepsize technique, both optimized for dealing accurately with planetary close approaches. Two different integrators were used to avoid any possible numerical artefact and to check the results. The dynamical model included all the planets except Pluto and Mercury, due to their small masses (the inclusion of Mercury would have also increased considerably the required integration times). The integration interval spanned at least from $t = -1$ Myr (in the past) to $t = 1$ Myr (in the future). In some cases we extended the integration up to 2.5 Myr in the past and/or the future, to find out the outcomes of clear secular trends in the elements.

Because of the strongly chaotic nature of typical NEA orbits, the results of the numerical integrations cannot be seen as deterministic predictions for the real objects over such long time spans, but provide only qualitative and/or statistical information on the most common patterns of orbital evolution and behaviour. This was confirmed by a few cases in which we integrated the same starting orbit with both numerical techniques quoted above: although the orbital evolutions were different (due to exponential increase of the small numerical errors), their qualitative behaviour was always similar. This is also the case when Mercury is included in the integrations: although close encounters with it become possible when the asteroid's eccentricity approaches unity, this provides only a small, stochastic additional source of perturbation. Thus, our integrations indeed refer to a sample of model objects which happen to have starting orbits coinciding (within the observational errors) with those of the corresponding real orbits. For the same reason, integrations backward and forward in time should be considered essentially equivalent to each other, and a solar collision found in the backward evolution does not necessarily mean that the corresponding real object is young.

The results shown in Table 1 can be summarized as follows: in the future, 16 of the 47 bodies have lifetimes < 1 Myr and of these, 12 collide with the Sun; in the past, the corresponding numbers are 13 and 9, respectively. Four more objects hit the Sun some time before or after ± 1 Myr. Apparently the Taurid-like bodies are more likely to hit the Sun (10 out of 21 in the

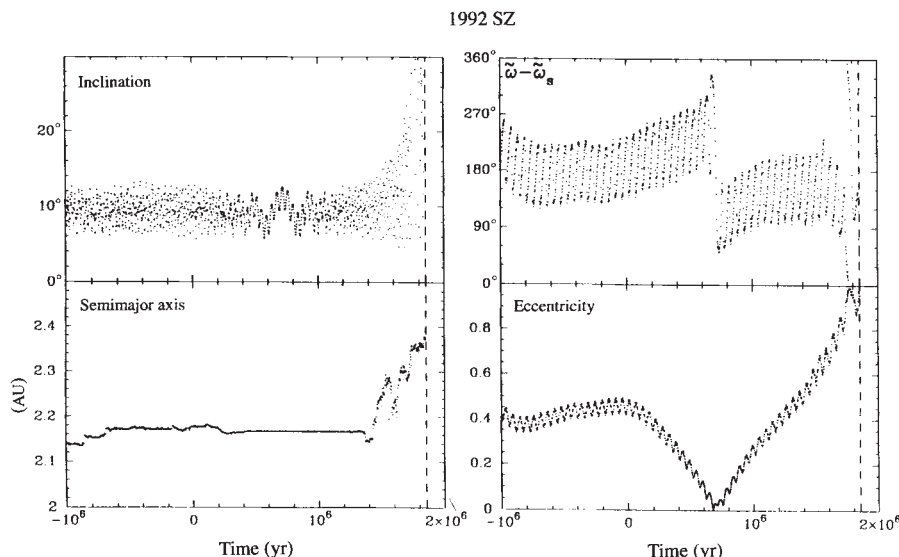
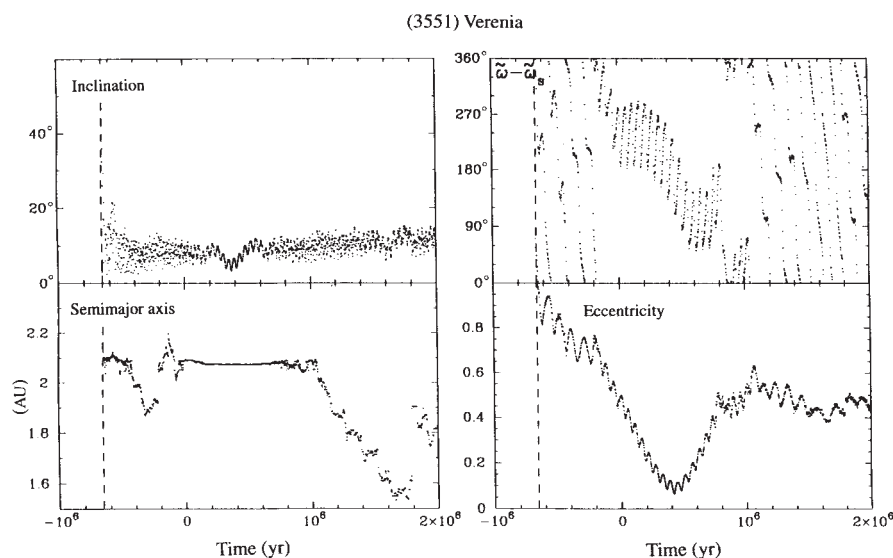


FIG. 1 Orbital evolution of the near-Earth asteroid 1992 SZ. The inclination, semimajor axis (a), critical argument of the ν_6 resonance ($\tilde{\omega} - \tilde{\omega}_s$, where $\tilde{\omega}$ and $\tilde{\omega}_s$ are the longitudes of perihelion of the asteroid and Saturn, respectively) and eccentricity (e) are plotted against time over the 2-Myr integration time span. The integration has been interrupted at $t = 1.86$ Myr, when the perihelion distance reaches 1 solar radius

(vertical dashed line). Note that the eccentricity almost vanishes at $t = 0.85$ Myr, and is subsequently pumped up rapidly by the ν_6 secular resonance. Other objects hitting the sun in our integrations with the same dynamical mechanism are (2212) Hephaistos (backward evolution), (4486) Mithra, 1983 LC, 1990 OA, 1991 BA (backward evolution) and 1991 TB₂ (both backward and forward evolution).

FIG. 2 As Fig. 1, but for asteroid (3551) Verenia. This object is currently in the ν_6 resonance, as shown by the libration of the critical argument $\tilde{\omega} - \tilde{\omega}_s$, with the eccentricity minimum to be reached at $t \approx 0.2$ Myr. In the future evolution close encounters with the Earth, starting at $t \approx 1$ Myr when $e \approx 0.6$, change a in such a way that the object exits from the resonance and stays at moderate eccentricity values. On the other hand, in the past the resonance pumps up the eccentricity to ~ 0.8 before encounters succeed in shifting the orbit outside the libration region—but at this point moderate secular oscillations of e (with arguments $\tilde{\omega} - \tilde{\omega}_j$, $\tilde{\omega} - \tilde{\omega}_s$ and $2\tilde{\omega} - \tilde{\omega}_j - \tilde{\omega}_s$, $\tilde{\omega}_j$ being Jupiter's perihelion longitude) are sufficient to cause the eccentricity to reach almost unity, and the perihelion 1 solar radius, at $t = -0.66$ Myr. Other objects hitting the Sun with a similar mechanism are (3833) 1971 SC, 1988 NE, (2101) Adonis, (2201) Oljato, (2212) Hephaistos (forward evolution), (4183) Cuno, 1984 KB, 1991 AQ, 1991 BA (forward evolution), 1991 GO and comet Encke (both forward and backward evolution).



future and 7 out of 21 in the past) than objects close to secular resonances (5 out of 26 in the future, 3 out of 26 in the past). This is probably due simply to the higher average starting eccentricity of the Taurid-like group, which appears to be just an advanced evolutionary stage of the near-resonant NEA population (G.B.V. *et al.*, manuscript in preparation). We found only eight cases of hyperbolic ejection overall. These results indicate that for this kind of orbit, a solar collision is significantly more likely than an ejection following a close planetary encounter. We detected no case of actual collision with a planet, in agreement with the fact^{13,14} that the characteristic time for such collisions is of the order of 10^8 yr.

It is remarkable that the number of solar collisions observed in our integrations is of the order of 10 per Myr, that is of the same order as the total loss rate estimated for NEAs > 1 km in diameter due to planetary impacts^{14,15}. But we integrated the orbits of only $\sim 25\%$ of the population of known NEAs, and of $\sim 2\%$ of the existing NEA population exceeding 1 km in size (some 2,000 objects overall, among which just $\sim 5\%$ are probably known). Although not all the NEAs belonging to our sample

are larger than 1 km, our results appear to imply that collisions of NEAs with the Sun are more frequent than both hyperbolic ejections (by a factor < 10) and planetary impacts (perhaps by an order of magnitude). This conclusion should hold in spite of the fact that our samples were biased: the NEA orbits that we integrated are the ones for which solar collisions on a comparatively short timescale were *a priori* most likely, because either they were close to a strong secular resonance, or they had large starting eccentricities. But the main effect of this bias is probably that of decreasing the dynamical lifetime with respect to solar collision rather than of increasing the overall chances of this eventual outcome: as the semimajor axes typically undergo a random walk due to planetary close approaches, most NEAs are likely to enter, sooner or later, one of the resonant routes responsible for solar collisions (as described below). Further numerical studies are needed to assess quantitatively this possibility and to estimate the fraction of existing NEAs on short-lived, Sun-hitting orbits compared to more long-lived, slowly evolving orbits (mainly affected by close encounters). Note that, owing to the different average lifetimes of the two

FIG. 3 As Fig. 1, but for asteroid (5731) 1988 VP₄. A solar collision happens at $t = 1.47$ Myr, with the orbit locked in the 3:1 mean motion resonance with Jupiter at $a = 2.50$ AU. At the same time, the inclination undergoes oscillations of large amplitude, between $\sim 10^\circ$ and 60° , as a consequence of the Kozai-mechanism coupling with the eccentricity¹⁷. The ν_6 critical argument $\tilde{\omega} - \tilde{\omega}_s$ alternates between clockwise circulation, libration (while the orbit is inside the 3:1 resonance) and counterclockwise circulation. At the beginning of the integration the orbit is locked in the 7:2 mean motion resonance with Jupiter (at $a = 2.25$ AU), but subsequently the semimajor axis is shifted by close encounters with the Earth. Other asteroids affected by mean motion and secular resonances are 1990 HA and 1991 VP₅. The former's orbit is also locked into the 3:1 mean motion resonance and at the same time is affected by the ν_6 secular resonance; in the latter's case, the ν_5 secular resonance inside the 5:2 mean motion resonance is responsible for the solar collisions. The 7:3 resonance may also play a similar role, as suggested by Yoshikawa's²⁰ numerical simulations of fictitious resonant objects.

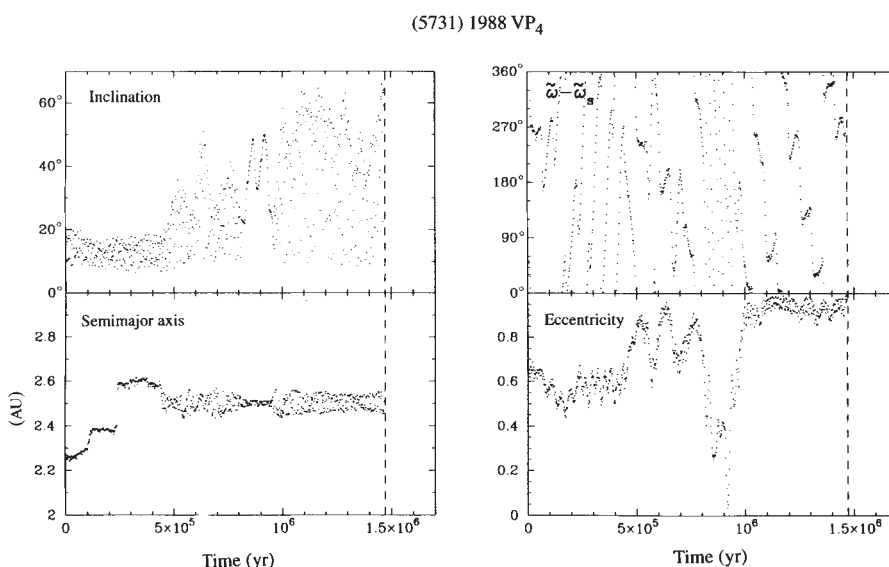


TABLE 1 Integrated objects and their fates

Number	Name	Past		Future	
		t_{Sun}	t_{hyp}	t_{Sun}	t_{hyp}
(3551)	Verenia*	-6.6			
(3883)	1971 SC*		-7.3	14.0	
(5620)	1990 OA	-9.6			
(5731)	1988 VP ₄ *			14.7	
	1988 NE			9.5	
	1991 VP ₅	-2.6		5.7	
	1991 SZ*			18.6	
(2101)	Adonis*			9.0	
(2201)	Oljato*			3.6	
(2212)	Hephaistos*	-10.2		1.1	
(4179)	Toutatis				6.4
(4183)	Cuno			1.8	
(4486)	Mithra		-5.5	1.8	
	1983 LC		-1.1	8.1	
	1984 KB	-2.7			
	1990 HA	-4.1			4.5
	1990 TG ₁		-6.5		4.2
	1991 AQ	-7.7			
	1991 BA	-3.8		1.2	
	1991 GO			6.0	
	1991 TB ₂	-2.5		0.3	
	6344 P-L				2.4
	Comet Encke	-3.8		0.9	

This Table lists the numbers and/or names, and the times of fall into the Sun (t_{Sun} , units 10^5 yr) or escape into hyperbolic orbit (t_{hyp} , units 10^5 yr) of asteroids having these fates during our integrations, which spanned at least from $t = -1$ Myr to $t = 1$ Myr (for bodies labelled by an asterisk, this was extended up to ± 2.5 Myr). The group in the upper part of the Table includes objects belonging to the sample of 26 NEAs with orbits close to the main secular resonances; in addition to those listed in the table, this sample included (512) Taurinensis, (1468) Zomba, (2368) Beltrovata, (3102) 1981 QA, (3402) Wisdom, (3671) Dionysius, (3858) 1986 TG, (4451) Grieve, (4596) 1981 QB*, (4924) Hiltner, (5646) 1990 TR*, (5690) 1992 EU, 1972 RB*, 1988 PA*, 1989 DA, 1991 VC, 1989 CC₁(=1992 PD), 1992 RD* and 1992 SY. The group in the lower part of the Table refers to NEAs with Taurid-like orbits; in addition to those listed in the Table, this sample included asteroids (4197) 1982 TA, (4341) Poseidon*, (5143) Heracles, 1990 SM and 1991 EE. The orbit of comet Encke, the likely parent body of the Taurid meteoroids, has also been integrated.

types of orbits, this fraction is necessarily lower than the fraction of main-belt asteroid fragments inserted originally in the 'fast-track' resonant routes and eventually hitting the Sun. As for the source of the NEA population, Farinella *et al.*⁷ have recently shown that disruptive impacts in the main asteroid belt may well inject into the resonances leading to Earth-crossing orbits some 100 fragments larger than 1 km per Myr, thus providing an adequate flux of new NEAs to balance the loss rate due to solar collisions.

We have identified three dynamical mechanisms that lead NEAs to hit the Sun, and these are illustrated in Figs 1–3. All of them are related to resonances. The first mechanism (Fig. 1) is simply the ν_6 resonance: as pointed out by Morbidelli⁴, the eccentricity can be pumped up to almost unity for orbits starting at semimajor axes between 2.1 and 2.3 AU, moderate inclinations, and even very low eccentricities.

A different mechanism is illustrated in Fig. 2, showing the evolution of asteroid (3551) Verenia. In this case the asteroid is removed from the ν_6 secular resonance by planetary encounters, but with such a high eccentricity value that subsequent moderate secular oscillations of the eccentricity are sufficient to cause a solar collision. In this case we have verified (by six more integrations of fictitious objects with starting orbital elements within 10^{-3} of the nominal ones for (3551) Verenia (corresponding to

relative velocities $\sim 10 \text{ m s}^{-1}$) that all of them end up hitting the Sun during the integration time span, so the evolution shown in Fig. 2 looks typical.

The asteroid (3551) Verenia is one of the few known V-type NEAs¹⁶, whose spectral features suggest a surface consisting of igneous rocks. Hence this NEA may be assumed to be a fragment of (4) Vesta, the only large main-belt asteroid which has a similar spectrum. In view of our results, it is instead tempting to speculate that past Sun-grazing episodes may be at the origin of the observed properties of (3551) Verenia. How many known NEAs (and meteorites) may have undergone such a thermal alteration process depends on the typical timescale between the first Sun-grazing episode and the eventual solar collision (or elimination by another mechanism), which is difficult to estimate from our integrations. As for meteorites, objects with eccentricities close to unity would hit the Earth at very high relative speeds, thus being preferentially eliminated during atmospheric crossing. Also, the optical properties of an asteroid or meteoroid surface having undergone a sequence of intense and short thermal pulses are not easily predictable, and should be investigated in the laboratory before trying to identify candidate NEAs for past Sun-grazing episodes from their spectra and/or albedos.

Figure 3 shows the third type of behaviour leading to a solar collision. Here the 3:1 mean motion resonance with Jupiter (with the asteroid revolving three times around the Sun while Jupiter completes one revolution) causes the eccentricity to jump from almost zero to >0.9 within <0.1 Myr. When the eccentricity is high enough, the Kozai-mechanism coupling¹⁷ with the inclination is also present. In this case, the ν_6 resonance shows up while the orbit is locked in the 3:1 mean motion resonance. In a few other cases, we have found that solar collisions can be the outcome of a chaotic region caused by a secular resonance inside a mean motion resonance, a dynamical mechanism understood only recently^{18,19}.

Our results suggest that solar collisions are relatively frequent not only for comets, but also for NEAs, and that several resonant routes are available to bring to the Sun a small body from the asteroid belt. Observational studies—and close-up space reconnaissance missions—may in the near future reveal hints of past Sun-grazing episodes for some existing NEAs, such as (3551) Verenia. Moreover, the existence of dynamical routes to the Sun needs to be taken into account both in current models of NEA evolution, and in estimates of the fragment flux from the main asteroid belt required to sustain a quasi-stationary NEA population. □

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Optical image processing by an atomic vapour

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ATOMIC vapours can exhibit large optical nonlinearities¹. When laser light is tuned in resonance with an atomic transition, the absorption cross-section of the atom can become very large, typically seven orders of magnitude larger than the cross-sectional area of its electron cloud². Because of these strong nonlinearities, different laser beams can interact with one another in an atomic vapour, even at intensities as low as a few milliwatts per cm². This raises the question¹ of whether atomic vapours can be used as nonlinear optical elements for parallel optical image processing. A well-known example of an all-optical image processor is the optical correlator: laser beams with imprinted images interact in a nonlinear medium to produce a signal beam, the intensity distribution of which is related to the correlation integral of (and hence the degree of similarity between) the input images. Here we demonstrate the use of a caesium-atom vapour as the active medium in such an optical correlator. We show that this system compares favourably with others currently in use, particularly with regard to its power requirements.

The interaction between light and free atoms finds interest and application in many different fields. The narrow transitions of free atoms have been used in the accurate definition of the time unit for decades. Atomic clocks constitute the foundation of the Global Positioning System which is becoming increasingly widely used, partly because of the availability of small hand-held devices³. Most of the past research in the information-processing applications of atomic vapours has concerned the switching of a single laser beam. Bistability⁴ and logic operations⁵ have been obtained. Here we report a demonstration of caesium-atom vapour as the active nonlinear material in an optical correlator, an example of the application of atomic vapours to highly parallel signal processing.

Caesium vapour provides resonant nonlinearities for infrared laser light at 852 nm (ref. 6). The laser source must have a small linewidth and be kept in resonance with the sharp absorption line. This requirement can be met by commercially available laser diodes (for example, model number SDL-5700, SDL Inc., San Jose, California). The caesium optical correlator operates with continuous-wave light at intensities of $\sim 10^{-3}$ W cm⁻², and exhibits the response time of 30 ns which one expects from the lifetime of the photoexcited atoms. As a comparison, fast response times have been obtained in other materials using high-intensity ($\sim 2 \times 10^6$ W cm⁻²) short light pulses at 10 Hz repetition rate⁷. This latter type of correlator requires bulky and expensive laser systems, and may be difficult to operate at a higher repetition rate. In contrast, because of its low-power operation at 852 nm, caesium vapour can in principle be used with commercial laser diodes.

A further advantage of atomic vapours for optical image processing is their flexibility. Nonlinear optical components of many different sizes and thicknesses can be easily built at low cost. A drawback is the fact that the atoms are not stationary. The movement of the atoms limits the minimum feature size that can be used inside the vapour to a diameter larger than ~ 40 μ m (ref. 1). Smaller feature sizes can be obtained by the addition of buffer gases at the expense of reduced sensitivity.

The best known example of optical information processing is the analogue calculation of a Fourier transform by a lens⁸⁻¹⁰. A coherent laser beam and a lens can be used to perform a two-dimensional Fourier transform as quickly as the light takes to

travel twice the focal length of the lens. This is much faster, though less accurate, than any digital Fourier-transform method. This property has sparked wide research interest for applications of optics in information processing¹¹. The advantage of optical computing and optical processing of two-dimensional images, as compared to electronic computers, resides in the massive parallelism of data transmission that is possible by imprinting the data on laser beams. Combinations of one- and two-dimensional Fourier transforms with lenses have been routinely used in the optical processing of synthetic aperture radar signals¹².

The optical Fourier transform can also be applied to the determination of correlation integrals between two images¹³⁻¹⁷. Two monochromatic images can be described by two complex electric field amplitudes $E_1(x, y)$ and $E_2(x, y)$. The correlation function E_c is defined as

$$E_c(x, y) = \int E_1(x + x', y + y') E_2^*(x', y') dx' dy' \quad (1)$$

where * denotes complex conjugation. In our present experiment, we use images that modulate only the amplitude of the laser beams, so that E_1 , E_2 and E_c can be considered to be real-valued functions.

The correlation function $E_c(x, y)$ assumes the highest value at the coordinates (x, y) where the two images are most similar. This property can be used to identify the position of a smaller image inside a larger image, or to find out if a certain image is present in a scene. For example, optical correlators can be used to inspect pills in the pharmaceutical industry¹⁸.

The possibility of implementing the correlation operation optically is evident if the integral in equation (1) is expressed in terms of the Fourier transforms of the images:

$$E_c = F^{-1}[F[E_1]F[E_2]^*] \quad (2)$$

where F and F^{-1} denote the Fourier transform and the inverse Fourier transform operations, respectively. The correlation function is obtained by calculating the Fourier transform of the two 'input' images, taking their product, and calculating the inverse Fourier transform of this product. The two-dimensional Fourier transforms can be easily obtained by using a lens. The correlation function can be optically calculated in the manner described by equation (2) if we have a way to obtain optically the multiplication of the two Fourier transforms. In our correlator the multi-

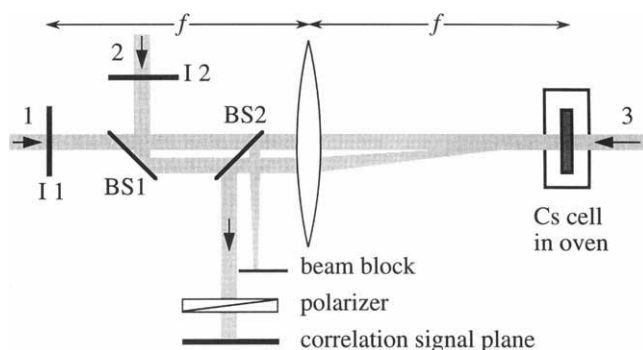


FIG. 1 Schematic of the optical correlator. Laser beams 1 and 2 pass through the image masks I1 and I2. The beams are then made parallel to each other by beam-splitter BS1 and are focused by the lens (with a focal length $f = 127$ mm). The Fourier transforms of the images overlap in the cell containing the caesium atoms. Laser beam 3, collimated to a diameter of 5 mm, interacts with the other two beams in the caesium-containing cell and produces the signal beam, which is Fourier-transformed by the lens, reflected by beam splitter BS2, and detected by the CCD array of a video camera. Beams 1 and 2 are polarized perpendicular to the plane of the figure. Beam 3 and the signal beam are polarized in the plane of the figure. The polarizer in the path of the signal beam suppresses undesired scattered light from beams 1 and 2.

plication operation is performed by a thin layer of a caesium-atom vapour confined in a glass cell.

A schematic diagram of the caesium optical correlator is shown in Fig. 1. Two input beams, collimated so as to approximate plane waves, pass through two masks creating beams with electric-field amplitude patterns $E_1(x, y)$ and $E_2(x, y)$. The Fourier transform of the two input images is obtained in the focal plane of the lens, where the cell containing the caesium atoms is located. A third beam, which also approximates a plane wave, interacts with the other two beams in the caesium cell and generates the signal beam.

The four-wave-mixing process that leads to the signal beam can be visualized by noting that the two input beams labelled 1 and 2 in Fig. 1 give rise to an interference pattern that modulates the optical properties of the vapour. This creates a diffraction grating of ground and excited states. Beam 3, which is counter-propagating to beam 1, is diffracted by this grating to produce the signal beam, which is then counterpropagating to beam 2. In a more quantitative description, the nonlinear interaction of the three input beams with the caesium atoms produces a pattern of dipoles proportional to the product of the complex amplitudes of the three input beams. These dipoles radiate the signal beam. The intensity distribution obtained at the output plane of the correlator is proportional to $|E_c(x, y)|^2$, the absolute square of the amplitude correlation function of the two images.

A caesium cell was fabricated by evacuating a 1-mm-thick Pyrex cell to $<10^{-6}$ torr. Caesium metal was introduced and the cell was then sealed. Heating the cell to 58°C in a temperature-stabilized oven produced an atomic density of 8×10^{11} caesium atoms per cm^3 , creating a linear absorption at line centre of the order of 70%. In such a cell we measured a ratio of 10^{-4} between

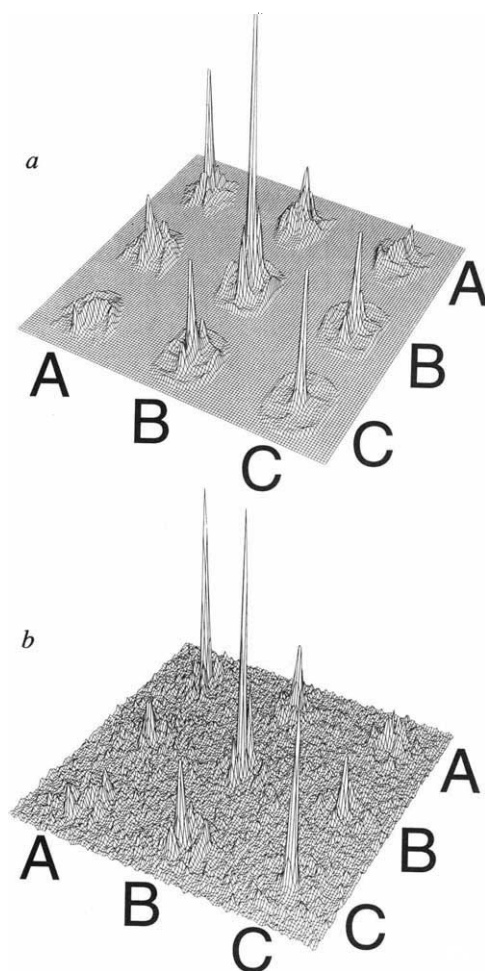
the power in the signal beam and the total input power. This was a standard four-wave-mixing experiment with two input beams counterpropagating to each other, the third input beam at a small angle of ~ 10 mrad, and a total intensity in the vapour of $\sim 1 \text{ mW cm}^{-2}$, shared equally between the three input beams.

In our experiments we use an external cavity frequency-stabilized Ti:sapphire laser operating at a wavelength of 852 nm with a line width of <1 MHz. The 1-mm-thick Pyrex cell contains $\sim 6 \times 10^9$ atoms in the interaction region (which has a diameter of ~ 3 mm). The Ti:sapphire laser is tuned to the centre of the cycling transition from the upper ground-state level $6S_{1/2}(F=4)$ (F denotes the total angular momentum of the atom, including nuclear spin) to the upper excited-state level $6P_{3/2}(F=5)$ (refs 6, 19). As explained by the selection rules for electric dipole transitions, atoms in this state can only decay back to the upper ground-state level from which they are photoexcited, thus avoiding the effects of optical pumping to the $6S_{1/2}(F=3)$ state^{6,19}.

As a first test of the quality of the caesium optical correlator, we used images of the letters A, B and C as an input, and measured the correlation function for all nine combinations of three letters in the two input images. The masks used in the input planes I1 and I2 (see Fig. 1) were amplitude masks obtained by photography on holographic plates. The input images, the theoretically expected correlation patterns, and the intensity distributions detected at the output plane of the caesium optical correlator are shown in Fig. 2.

We calculated the absolute square of each of the cross-correlation functions of the three images representing the letters A, B and C, using equation (2) on a computer. The results are shown in Fig. 2a. The columns and rows in the Figure corresponding

FIG. 2 Cross-correlation table of images of the three letters A, B, C. The columns and the rows in the plot are labelled with A, B and C. The correlation function of image 1 and image 2 is found in the first row and second column, and in the second row and first column. The peaks on the diagonal correspond to equal images being input into the correlator. *a*, Mathematical calculation of the expected intensity pattern at the output of the correlator. The square $|E_c(x, y)|^2$ of the amplitude correlation function is plotted as a function of the x, y coordinates. *b*, Combined results of nine experiments. The letters on the masks are $230 \mu\text{m}$ tall. The input power on beam 1 is $80 \mu\text{W}$ and the input power on beam 2 is $420 \mu\text{W}$. Beam 3 passes through a 3-mm aperture and has a power of $40 \mu\text{W}$. The intensity corresponding to the peak of the autocorrelation of C is $0.27 \mu\text{W cm}^{-2}$. The powers of the two image-bearing beams given here are the ones that can be measured just before beam splitter BS2 (see Fig. 1). The intensity of the signal beam is the one just after beam splitter BS2.



to a given letter are labelled with that letter. The autocorrelation functions are seen on the diagonal.

The signal detected with the CCD (charge-coupled device) array of a commercial video camera at the output of the optical correlator is shown in Fig. 2b, scaled to the same dimensions as the mathematical result of Fig. 2a. The various correlation functions of the nine different letter pairs were measured separately and combined to obtain Fig. 2b. The fidelity of the experimentally determined correlation functions to the mathematically calculated correlation functions is demonstrated. The total power in the autocorrelation peak of the letter C is 5×10^{-12} W (5 pW) while the total input power is 540 μ W. The size of the letters on the masks is ~ 200 μ m. The width of the correlation peaks is given by the thickness of the lines used to draw the letters, which is 35 μ m. The caesium optical correlator can respond to new images in 30 ns, the lifetime of the photo-excited caesium atoms. The combination of its low-power operation and its fast response make caesium vapour a candidate for the most sensitive material demonstrated in a correlator to date¹. A more detailed experimental analysis of the caesium optical correlator will be published elsewhere.

The caesium optical correlator compares favourably to a recently demonstrated correlator using a photorefractive multiple-quantum-well device¹⁷. The latter uses two laser diodes at different wavelengths and has a response time of the order of 1 μ s. The caesium optical correlator has the advantage of requiring much less light intensity (in excess of an order of magnitude), and requiring only a single wavelength, while allowing a faster switching time.

With either the multiple-quantum-well correlator or the atom correlator reported here, the capacity of a practical image processor would be limited presently by existing spatial light modulators and output analysers. Spatial light modulators that can display and erase 256×256 pixel images within 100 μ s have been reported²⁰. To obtain a table of the coordinates of all correlation peaks larger than a certain threshold would seem at present to take more time, and may be considered to be the bottleneck in the process²¹.

The optical correlator can be regarded as a step towards more diverse applications, such as matrix multiplication or pattern recognition, that might be part of a more complex optical processor. A major requirement is the availability of adequate non-linear optical materials. With our atomic vapour correlator we have demonstrated a new material that can be considered for parallel optical processing, a material as simple as an atomic vapour enclosed inside a thin glass cell. $\square$

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Photochemical closing and opening of the guest-binding cavity of cyclodextrins

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CYCLODEXTRINS and modified derivatives can bind, and sometimes modify the properties of, guest molecules in their torus-shaped cavities^{1,2}. They have also been used as the building blocks of molecular materials and devices³. The propensity to bind and retain a guest is not easily predictable or controllable, however. There is currently much interest in the switching on and off of chemical⁴ and biological⁵ activity, particularly by photochemical means⁶, as such functions will be required of molecular-scale devices. Here we report the controlled binding and release of guest molecules in cyclodextrins modified with substituents that can reversibly form bridging units across the cavity openings. Irradiation of percinna-moylated α - or β -cyclodextrin in *N*-methylpyrrolidin-2-one (NMP) leads to the formation of intramolecular cyclobutane bridges which trap a bound NMP molecule. Irradiation at a different wavelength breaks open the cyclobutane rings and releases the guest.

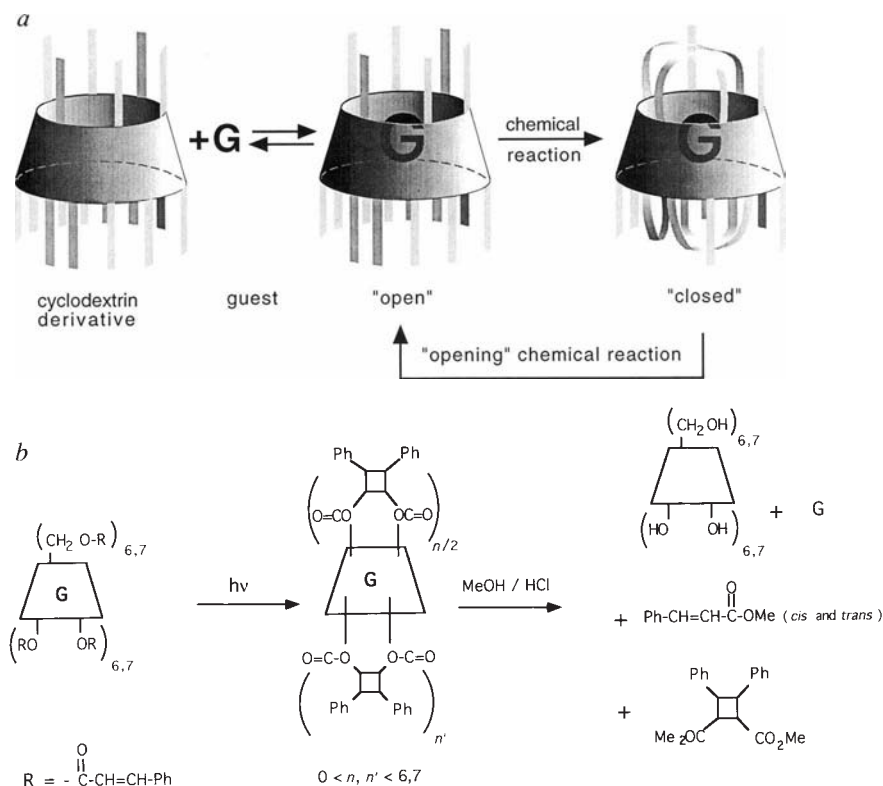
All the hydroxyl groups in α -cyclodextrin (α -CD) and β -CD were esterified with cinnamic acid residues to obtain suitable derivatives for testing the notion of closing the top (primary hydroxyl) and bottom (secondary hydroxyl) openings of cyclodextrins (CDs) and imprisoning guest molecules present during the closure reaction. As di- and polycinnamates undergo efficient intramolecular C=C double bond (2+2) photocycloaddition reactions to yield cyclobutane rings⁷, it was expected that the bulky cyclobutane units (truxinic esters) would bridge across the top and bottom rims of the CD molecules and close off the exterior environment, forming a closed CD cavity. Furthermore, because such cyclobutane rings may be broken by short-wavelength irradiation, it was anticipated that guest release could also be demonstrated (Fig. 1).

By treating the appropriate CD with excess cinnamoyl chloride in pyridine⁸, α -cyclodextrin percinamate (α -CD-PC) and β -CD-PC were obtained. The purified (column chromatography on silica gel) products were isolated as microcrystalline solids having ¹H and ¹³C NMR spectra in full accord with peracylated (six- and seven-fold, respectively) time-averaged symmetrical structures (three non-equivalent cinnamate signals). Mass spectral analysis afforded the expected protonated molecular ion, MH⁺ values. The percinamates are soluble in organic solvents such as chloroform, methylene chloride, acetone, acetonitrile and *N,N*-dimethylformamide but insoluble in water, protic (methanol, ethanol, 1- and 2-propanol) or hydrocarbon (*n*-hexane, *n*-octane, iso-octane) solvents.

When deaerated solutions of α -CD-PC or β -CD-PC were irradiated (Pyrex-filtered light from a xenon-mercury lamp), the 276-nm absorption due to the cinnamoyl chromophore (Ph—CH=CH—CO—) diminished with time, and the infrared bands at 1,721 and 1,636 cm⁻¹ (CO and CH=CH, respectively) were replaced by a new band at 1,751 $\pm$ 1 cm⁻¹, expected for the aliphatic ester C=O present in the cyclobutane (truxinate) photoproducts. For preparative reactions, deaerated solutions were irradiated in a Rayonet reactor fitted with lamps having strong emission at $\sim$ 300 nm. A number of solvents which are potential CD guests were used; irradiated solutions of ethyl acet-

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FIG. 1 a, Schematic view of the reversible inclusion and imprisonment of a guest molecule, G, by a cyclodextrin (CD) derivative and subsequent closure of the top (primary hydroxyl side) and bottom (secondary hydroxyl side) through intramolecular chemical linking of the CD substituents, thereby trapping the guest. An appropriate (different) chemical reaction opens the sealed CD and releases the guest. b, Intramolecular photocycloaddition reactions using percinamoylated CDs. Following irradiation (300-nm light) and acid-catalysed ester exchange in methanol, the cyclobutane products shown, which constitute the bridges that lock in the guest, were obtained. Photocleavage (254-nm light) of the cyclobutane groups in the bridged product also releases the guest, G. The depicted cage photoproduct represents an ensemble of structures containing varying numbers of cyclobutane rings and *cis*- and *trans*-cinnamate residues.

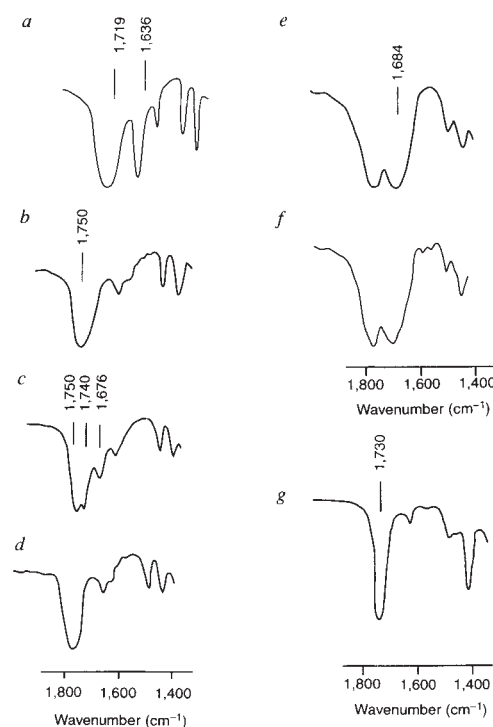


ate and *N*-methylpyrrolidin-2-one (NMP) were studied in more detail.

After irradiation (~120 h), the cyclodextrin-containing material was isolated by evaporation of the solvent (ethyl acetate) or by precipitation with methanol (NMP). The solids were redissolved in methylene chloride and precipitated with methanol. Centrifugation and removal of supernatant left solids which were triturated with methanol and centrifuged again. Finally, the solid products were warmed to ~100 °C for 1 h at reduced pressure (1 mm Hg). When unirradiated α -CD-PC or β -CD-PC was dissolved in NMP and treated in this fashion, the characteristic infrared band of NMP (at 1,676 cm⁻¹) was absent from the samples (Fig. 2a). Similarly, when the product from an irradiated ethyl acetate solution of α -CD-PC or β -CD-PC was dissolved in NMP and purified according to this protocol, the product contained no NMP (Fig. 2d). On the other hand, when a solution of α -CD-PC or β -CD-PC in NMP was irradiated and subjected to this procedure, the final product showed clear evidence for the presence of NMP: strong infrared bands, and clear peaks in mass spectra (Figs 2e and 3b). Even prolonged heating under more drastic conditions (~200 °C) affords an

unchanged product sample containing NMP (Fig. 2d). The C=O band (1,684 cm⁻¹) in the infrared spectrum of complexed NMP in the 'locked' CD cage is shifted relative to uncomplexed NMP (1,676 cm⁻¹); this is observed in both solution (chloroform) and solid matrices (KBr) and is consistent with a guest restricted to the less polar environment of the CD interior where hydrogen-bonding is less favourable. Further evidence for guest entrapment in a closed CD cavity was obtained by mass-spectral analysis of the samples before and after irradiation as a function

FIG. 2 Infrared spectra (Nicolet 510 Fourier transform infrared spectrometer; KBr pellets) of: a, β -CD-PC, before irradiation; b, β -CD-PC, after irradiation in ethyl acetate and work-up as described in text. The bands at 1,636 and 1,721 cm⁻¹, associated with the CH=CH and C=O absorption of the cinnamate, have decreased and a new band at 1,750 cm⁻¹ appears; c, sample b after dissolving in NMP and re-isolation without heating in vacuum (traces of NMP are present, as shown by band at 1,676 cm⁻¹); d, same as sample c but heated for 1 h at 100 °C at a pressure of 1 mm Hg; no trace of NMP is indicated but the bands at 1,740 (ethyl acetate C=O) and 1,750 cm⁻¹ here and in spectrum c are unchanged; e, β -CD-PC, after irradiation in NMP and work-up as described in text; the strong band at 1,684 cm⁻¹ is ascribed to trapped NMP; f, same as sample e, but heated for 2 h at 200 °C at a pressure of 1 mm Hg; no change from e is noted; g, sample as in e after dissolution in ethyl acetate, irradiation in a quartz tube (254-nm wavelength light; 120 h) and work-up as before; this spectrum was recorded in chloroform and indicates complete release of guest on irradiation.



of sample warming (Fig. 3). For all of the guests studied, the thermal release, as detected by appearance of the guest signals in the mass spectrum, only takes place when the complex is heated to $\sim 350^\circ\text{C}$. By contrast, adsorbed guest or guest included in unirradiated CD-PC is released directly at room temperature (Fig. 3a).

Irradiation of the closed β -CD-PC.NMP complex in ethyl acetate or in acetonitrile at a wavelength (254 nm) which is known to photochemically split aryl cyclobutane rings⁹, followed by precipitation with methanol and the standard work-up, afforded product lacking the characteristic guest NMP infrared band at $1,684\text{ cm}^{-1}$ (Fig. 2g). Thus the sealed CD cavity may be opened, and the guest released, by irradiation with short-wavelength light.

Because of the dilute conditions used ($(1-5) \times 10^{-4}\text{ M}$), the photochemical reactions of the percinamoylated CDs were expected⁷ to be largely, if not exclusively, intramolecular; thin-layer chromatography corroborates this (very similar R_f mobility values for the unirradiated and irradiated samples). When a purified, irradiated β -CD-PC sample was treated with methanol/HCl to effect ester exchange, methyl cinnamate (*cis*- and *trans*-isomers) and dimethyl esters of cinnamate photodimers were obtained (Fig. 1b). The major dimers are β - and δ -truxinate (1:4, $\sim 90\%$ of the total; identified by gas-liquid chromatography (GLC) comparison with authentic samples and NMR of the purified reaction mixture) as well as other truxinates that seem to be the product of *cis* + *trans* photoaddition. The ratio of photodimer to cinnamate, and the decrease of the cinnamate

ultraviolet absorption, indicate that $\sim 80\%$ of the cinnamates have reacted.

Many minimum-energy CD-PC structures possess the close cinnamate-cinnamate contacts required for 2 + 2 photocycloaddition reactions; their photoproducts (carcerands¹⁰) cannot be formulated by a single structure as they are ensembles of many isomeric molecules. Molecular modelling of α -CD-PC (data not shown) revealed conformations having two cinnamate residues on the primary face in good juxtaposition for cyclobutane ring formation; the resulting cyclobutane photoproduct effectively closes the top of the CD cavity. Close O2-O3' cinnamate-cinnamate interactions (adjacent glucose rings) in β -CD-PC appear in many minimized conformations; the photoformation of two cyclobutane bridges from such pairs essentially closes off the bottom (secondary hydroxyl) rim of β -CD. Because many conformations are possible and the energy differences between them are small ($\sim 0.5\text{ kcal mol}^{-1}$, ref. 7), it is difficult at this stage to ascertain which of the many possible photoaddition reactions are more favourable or which are critical for guest entrapment. Current studies with blocked derivatives may elucidate this point.

Bridging across cyclodextrins and other host molecules has been studied by a number of researchers^{11,12}, but the formation of closed-surface hosts by intramolecular reactions has not been previously described. The cyclodextrin percinamates, related to other acylated CDs¹³, provide an especially easy approach to closed-surface cavities, termed carceplexes^{10,14}, and to switchable states using photochemical reactions. Non-photochemical intramolecular closure is an important extension of the scheme depicted in Fig. 1.

The 'inner phase' of the molecular container compounds that Cram and co-workers have designed and studied were termed by him "a new phase of matter" which may be used, for example, to isolate otherwise unstable species¹⁴. Depending on the volume of the enclosed CD cavity and the geometries and volumes of the guests, more than one guest, of the same or different nature, may be encapsulated and otherwise unattainable guest-guest interactions, and chemical transformations, may thus become possible (infrared data, not shown, indicates that the cage products of irradiated β -CD-PC in NMP contain more than one NMP molecule). For this application γ -CD-PC should be more versatile; furthermore, substituents that extend the cage size may be considered for the design of different cavity shapes and sizes. By suitable choice of the chromophore (such as substituted cinnamates) the wavelength of irradiation needed to open and close the CD-cages can be controlled. Indeed, many different closing functionalities may be considered, for reversible (or irreversible) opening, and for tailored applications, such as the incorporation of enzyme-selective bridges for tissue-specific targeting and release of drugs. □

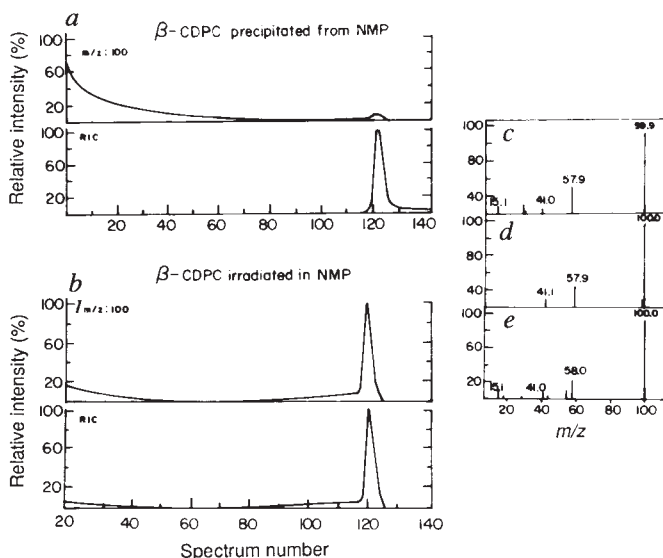


FIG. 3 Mass-spectral analysis of β -CD-PC before (a) and after (b) irradiation in NMP. The insert probe of a PGS-70B Finnigan-Mat instrument was kept at room temperature for 60 s, and then heated to 400°C over a period of 120 s. Spectra were recorded at 1.5 s intervals. Both total ion, recombined ion current, RIC, for (CD + guest) and prominent guest ions (m/z 100, MH^+ for NMP; for ethyl acetate, data not shown: m/z 88) were monitored, and the intensity of each peak plotted as a function of spectrum number. a, Nearly all of the ions appear at room temperature and at the early stages of warming; these are due to the adsorbed or included NMP in this sample which was dried in vacuum at room temperature but not heated. d is the composite of spectra 17–24 from sample a and is the same as c, the spectrum of authentic NMP, taken under identical conditions. The peak at spectrum number ~ 125 ($\sim 375^\circ\text{C}$) is believed to be due to disintegration of β -CD-PC. The small peak in the plot of the intensity of the m/z 100 line, at spectrum number 125, is due to traces of NMP and other species. Assuming identical sample conditions, this peak is $<5\%$ of the NMP peak in b. b, Nearly all the ions appear at $\sim 375^\circ\text{C}$ (spectrum number 120 corresponds to this temperature); e, the spectrum of the released molecules (combined spectra 118–121 of sample b; spectra 94–107 were subtracted to eliminate noise) and is seen to be NMP; the combined spectra 121–128 contain NMP and other ions ascribed to disintegration of β -CD-PC.

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The role of the deep ocean in North Atlantic climate change between 70 and 130 kyr ago

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THE suggestion¹ that changes in North Atlantic Deep Water (NADW) production are linked through surface heat flux to the atmospheric temperature over Greenland is supported by earlier indications^{2,3} that NADW production decreased during glacial times, and by the subsequent finding^{4–6} that it declined during the Younger Dryas cool period at the end of the last glaciation. Changes in North Atlantic surface temperatures have been found⁷ to mirror high-frequency temperature changes recorded in Greenland ice cores over the past 80 kyr, but the connection to abyssal circulation has yet to be established, except for one or two isolated oscillations^{8,9}. Here we present carbon and oxygen isotope analyses of benthic foraminifera in a high-resolution North Atlantic deep-sea sediment core for the period 70–130 kyr ago. These data allow us to reconstruct the history of NADW production, which shows a close correlation with Greenland climate variability for much of this time interval, suggesting that the climate influence of NADW variability was widespread. We see no evidence, however, for changes in NADW production during substage 5e (the Eemian interglacial period), in contrast with recent ice-core data¹⁰ which suggest severe climate instability in Greenland during this time period. Our results may support suggestions, based on data from a second ice core, that this apparent instability is an artefact caused by ice flow¹¹. Alternatively, the Eemian climate instability may have had a different origin from the subsequent climate events.

We chose piston core Knorr 31 GPC9 for a detailed time-series study. This long, large-diameter core was recovered from a depth of 4,758 m in a sediment wave field on the west flank of the Bahama Outer Ridge. The ridge is a constructional deposit, moulded by deep contour-following currents¹²; modern bottom waters at the core location contain ~15% recirculated Antarctic Bottom Water (AABW) mixed with NADW¹³. Because of its western boundary location, GPC9 is ideally positioned to monitor the chemistry of waters exported from the Atlantic basin, and its water depth makes it very sensitive to changes in the balance between NADW and AABW in the deep North Atlantic.

Oxygen isotope analyses of the benthic foraminifera *Cibicides* spp. and *Nuttallides umbonifera* provide a standard sequence of maxima and minima which are readily identified as the substages of isotope stage 5 (Fig. 1a). Eight levels have been assigned ages according to an orbitally-tuned and globally-stacked age model¹⁴. Average rates of sedimentation derived from this age model for stage 5 at GPC9 are 5.6 cm kyr⁻¹, although rates of sedimentation may be much higher during episodes of harsher climate⁹. Between ~135 and 125 kyr ago $\delta^{18}\text{O}$ values decreased by ~2‰ (Fig. 2a), reflecting both the decrease in continental ice volume which produced a high stand of sea level during isotope substage 5e and the warming of deep waters in the Atlantic Ocean¹⁵. The substage 5e interglaciation, which has been previously correlated to the Eemian on land¹⁶, lasted ~10 kyr and was followed by falling sea level and two intervals of equable climate (substages 5c and 5a) interrupted by two intervals of harsher climate (substages 5d and 5b).

In general, $\delta^{13}\text{C}$ results anticorrelate with $\delta^{18}\text{O}$ (Figs 1b, 2b). During peak interglacial (substage 5e) and interstadial (substages 5a and 5c) conditions, maximum $\delta^{13}\text{C}$ is achieved due to a combination of increased terrestrial biomass^{17,18} (which might account for about a third of the signal in GPC9) and increased production of NADW. Today, NADW typically has $\delta^{13}\text{C}$ values of ~1‰ (ref. 19), although no data exist from near core GPC9 where the influence of AABW would be expected to lower the values. Thus, the values of 1‰ observed during substage 5e support the interpretation of Duplessy and Shackleton²⁰ that NADW production was then near modern levels. Values during glacial stage 6 and leading into glacial stage 4 were as low as -0.5‰, equivalent to $\delta^{13}\text{C}$ in the Southern Ocean during the maximum of the last glaciation^{18,21}. This implies that the deep western North Atlantic experienced a substantial decrease in the ratio of NADW to AABW during glacial stages 4 and 6.

Our most significant new finding in GPC9 is that $\delta^{13}\text{C}$ results display variability at significantly higher frequencies than seen

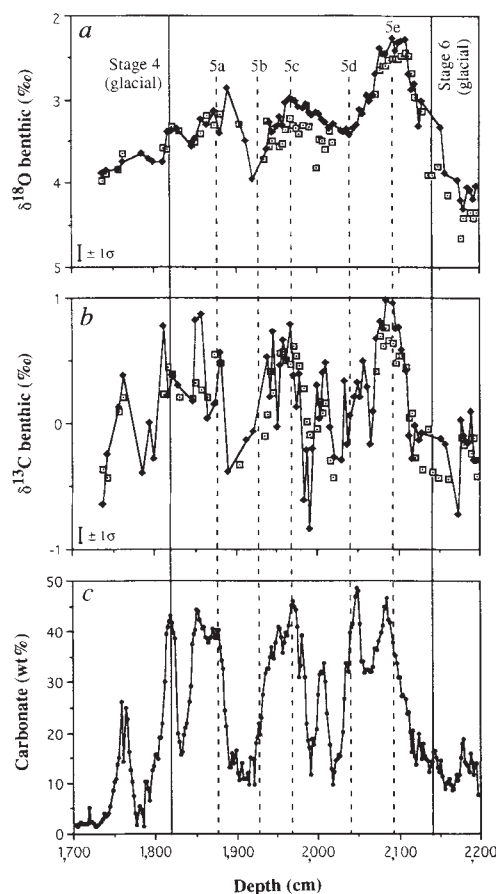


FIG. 1 Oxygen isotope (a), carbon isotope (b) and wt% CaCO_3 (c) from western North Atlantic core KNR31-GPC9 (28° 14.7' N, 74° 26.4' W, 4,758 m). Stable isotope ratios were measured on the benthic foraminifera *Cibicides* spp. (solid symbols) and on *N. umbonifera* (open symbols) with an analytical uncertainty (in standard analyses) represented by the small bar in the lower left corner of panels a and b. For $\delta^{13}\text{C}$ data, the signal-to-noise ratio is 15:1. Each displays a similar pattern of changing continental ice volume and deep ocean temperature, which is used to assign the boundaries of isotope stages (solid vertical lines) and the position of stage 5 extrema (dashed vertical lines; a). These foraminifera also reveal the same general pattern of high $\delta^{13}\text{C}$ during warm substages 5a, c and e, with lower values during cooler substages 5b and d (b). Ignoring occasional one-point peaks, note that CaCO_3 and $\delta^{13}\text{C}$ results bear a striking resemblance, reflecting the common origin of these signals in deep ocean circulation patterns.

in $\delta^{18}\text{O}$ (Fig. 2). The brevity of the $\delta^{13}\text{C}$ oscillations rules out forcing by the ice-sheet-driven, continental biomass effects suggested by Shackleton¹⁷ because significant continental ice accumulation takes many millennia. Instead, the millennial-scale variability is probably dominated by abrupt and oscillating changes in the flux of NADW. Significant $\delta^{13}\text{C}$ changes occur

without regard for the background state of the climate system; high production rates of NADW occur during both interstadial and stadial conditions. For example, although $\delta^{13}\text{C}$ initially declined early in glacial substage 5d as is typical at other locations^{20,22}, it was quickly followed by a prominent $\delta^{13}\text{C}$ maximum ~ 113 kyr ago, and then another minimum (Fig. 2b). Conversely, ~ 103 kyr ago in interstadial substage 5c there was a $\delta^{13}\text{C}$ minimum which indicates NADW suppression comparable to full glacial times. In all, there appear to be three maxima and minima in $\delta^{13}\text{C}$ for each precession cycle seen in $\delta^{18}\text{O}$.

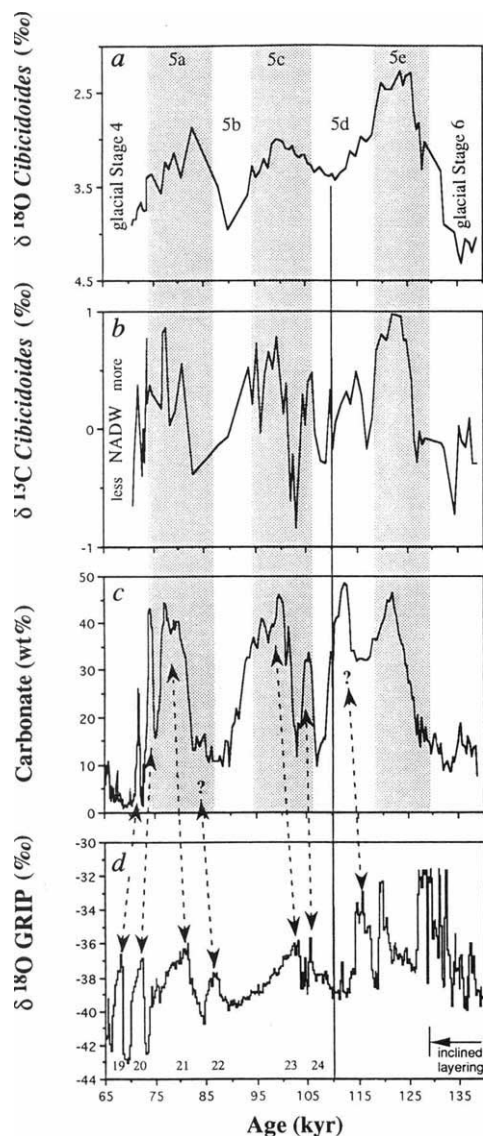


FIG. 2 *Cibicidoides* stable isotope data (a, b) and wt% CaCO_3 (c) from Fig. 1 compared to the $\delta^{18}\text{O}$ of the GRIP ice core from Summit, Greenland (d). GPC9 data are plotted versus age using $\delta^{18}\text{O}$ stratigraphy (Fig. 1a) and the Martinson *et al.*¹⁴ chronology. Warm interglacial substages in the sediment core are shaded, and interstadials in the ice core are numbered. The ice-core chronology was discussed by Dansgaard *et al.*²³ and is pinned to the deep-sea chronology at the 110-kyr level, denoted by the solid vertical line. Both GRIP and GISP2 records correlate, and are thought to be reliable back to interstadial 23, but the deeper occurrence of inclined layering at GRIP suggests that it may have a record that is reliable as far back as ~ 129 kyr ago¹¹. As discussed in the text, $\delta^{13}\text{C}$ variability in GPC9 probably reflects changes in the relative proportion of North Atlantic Deep Water and Antarctic Bottom Water, and CaCO_3 variability likewise reflects changes in thermohaline circulation. Changes in thermohaline flow and its associated influence on meridional heat flux of the surface North Atlantic are probably linked to atmospheric temperature over Greenland for events indicated by short dashed arrows.

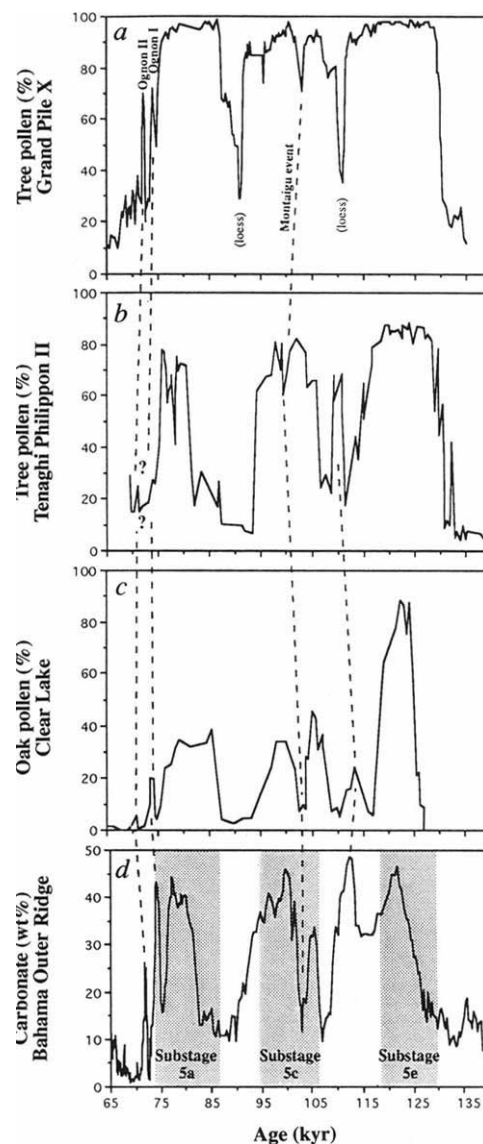


FIG. 3 Correlation of millennial-scale climate events in the North Atlantic, using wt% CaCO_3 in GPC9 as a proxy for deep ocean circulation change (d), with pollen records from Grand Pile in France²⁴ (a), from Tenaghi Philippon in Macedonia²⁹ (b), and from California²⁶ (c). We have applied the SPECMAP age model¹⁴ to the pollen data using correlations proposed by the original authors. Shaded intervals in the GPC9 panel mark warm substages of interglacial isotope stage 5. Dashed lines correlate the Ognon I and Ognon II warm events of ~ 70 – 75 kyr ago, the cold Montagu event which occurred ~ 103 kyr ago, and a warm event within stage 5d (~ 113 kyr ago). We propose that these and other short duration events in palaeoclimate proxy data are global, and may be related to brief changes in North Atlantic thermohaline circulation.

Carbon isotope changes in benthic foraminifera closely parallel the variability in the concentration of CaCO_3 (wt%) in GPC9 (Figs 1 and 2). Carbonate analyses provide a smoother, more detailed series of data than $\delta^{13}\text{C}$ because (not being subject to the availability of foraminifera) they can be performed on small bulk samples. The $\delta^{13}\text{C}$ - CaCO_3 relationship has been observed previously for a few millennial-scale oscillations in the last 80 kyr of GPC9 as well as core KNR31-GPC5 from the northeast Bermuda Rise⁹. The large changes in sediment carbonate reflect mostly terrigenous input to the North Atlantic during glaciation, its redistribution by deep recirculating gyres (that is, the strength of thermohaline circulation), and CaCO_3 dissolution. As NADW (^{13}C -enriched) is less corrosive to CaCO_3 than AABW (^{13}C -depleted), replacement of NADW by AABW drives down percentage CaCO_3 . Thus, both the records of wt% CaCO_3 and $\delta^{13}\text{C}$ in deep western North Atlantic cores respond to high-latitude thermohaline processes, accounting for their similarity.

Considering the importance of NADW production in maintaining the meridional flux of heat at the surface of the North Atlantic¹, it is appropriate to ask how our proxy data for deep ocean circulation change compare to the $\delta^{18}\text{O}$ data (an atmospheric temperature proxy) from the new Greenland ice cores. We focus on the GRIP core (Fig. 2d) because it appears to have a longer undisturbed record than the GISP2 core¹¹. Age assignments for GRIP data are based on ice flow modelling, and correlation to marine isotope substage 5d²³ (Fig. 2d).

Younger than ~110 kyr, GRIP $\delta^{18}\text{O}$ results compare well with our proxy for NADW production within accepted dating uncertainties (a few thousand years) in each proxy record (Fig. 2). As noted previously for both core GPC9 and Bermuda Rise core GPC5, two maxima in $\delta^{13}\text{C}$ and wt% CaCO_3 near the stage 5a/4 transition are equivalent to isotopically warm events in Greenland and Antarctic ice cores, as well as European pollen records⁹. These events are clearly recorded in the GRIP core as interstadials 19 and 20. Interstadial 21 is equivalent to the peak of isotope substage 5a, although interstadial 22 is not readily identified at core GPC9. (That event may not be recorded because of a 10-kyr interval of especially low benthic foraminiferal abundance that prevented isotope analysis of all samples.) Marine isotope substage 5c is marked by a prominent double maximum in the NADW proxy data, and those data appear to correlate well with interstadials 23 and 24 in the ice core. Correlations older than substage 5d could be complicated by uncertainties in the ice-core record, but the presence of maxima in wt% CaCO_3 and $\delta^{13}\text{C}$ within substage 5d (~113 kyr ago) in GPC9 suggest that a strong warming may have followed peak interglacial conditions. How that event and the maximum in $\delta^{13}\text{C}$ that occurred during substage 5e at GPC9 correlate with the three oxygen isotopic maxima in the GRIP record remains inconclusive. We speculate that the warm event seen in the ice cores at ~115 kyr ago (Fig. 2d) may correlate with the $\delta^{13}\text{C}$ and wt% CaCO_3 maxima in substage 5d at GPC9. There is no evidence for deep ocean variability within substage 5e, implying that ice-core variability at that time must result from some other process.

An additional test of the link between NADW production, North Atlantic heat flux and air temperature over Greenland is provided by comparison to other climate indicators. In particular, it is useful to compare our marine record to pollen records that display previously unexplained variability at frequencies much greater than orbital ones. The events punctuating the stage 4 glacial inception in western North Atlantic cores and Greenland ice cores appear to correlate with the warm events labelled as Ognon I and Ognon II at the Grand Pile peat bog in France²⁴ (Fig. 3). Recently it was reported from detailed study of a second Grand Pile core that only one Ognon interstadial is clearly recognizable, and that the other event is probably due to pollen reworking²⁵. A similar pair of events is also found in the Vostok ice core in Antarctica⁹, and now we extend that correlation to Clear Lake, California (Fig. 3c), which to our knowledge con-

tains the only continuous high-resolution pollen record reaching to the peak of the last interglaciation in North America²⁶. Considering that the double events leading into stage 4 are evident in several Greenland sites, in Antarctica, in North America as well as in two high-resolution sediment cores from the North Atlantic, we conclude that these events are probably global in scope and somehow related to changes in formation rate of NADW.

Other high-frequency events from within the standard isotope stage stratigraphy are also found consistently among the most complete and best resolved palaeoclimate records. The isotopically cold event between interstadials 23 and 24 in the GRIP core, which corresponds to the $\delta^{13}\text{C}$ (Fig. 2b) and CaCO_3 minima at GPC9 at ~103 kyr ago (Figs 2c and 3d), is known from Grand Pile as the 'Montaigu event'^{24,27}. That event is coherent among several French pollen sequences^{27,28}, and is present as well at Tenaghi Philippon in Macedonia²⁹ and at Clear Lake²⁶. It is also possible to correlate the Montaigu event with a cooling ~103 kyr ago in Vostok deuterium data, using the new SPECMAP age model for that core³⁰. Finally, we note that both Tenaghi Philippon and Clear Lake pollen reveal a warming that may correlate with the CaCO_3 and $\delta^{13}\text{C}$ maxima within substage 5d at GPC9. A similar warming is not evident in Grand Pile pollen sequences, perhaps because the substage 5d correlative (Melisey I) is represented by only a short section of core which is dominated by loess sedimentation³¹. We concede that these correlations are subjective and based on curve-matching, but previously there was no explanation for many of these high-frequency climate shifts. Here we provide the first evidence that these events may have been caused by changes in deep ocean circulation.

These deep ocean changes also recur more frequently than the intervals of accelerated iceberg discharge known as Heinrich Events⁷, suggesting that processes other than (or in addition to) mechanical destabilization of large ice sheets modulated NADW flow. Although we cannot yet determine exactly what factors governed high-frequency variations in NADW production during stage 5, the most likely candidates are changes in meltwater flux and/or the balance between evaporation and precipitation over the high-latitude North Atlantic. The fact that we do not detect changes in NADW during substage 5e (Eemian), a time when there was less continental ice than now, suggests that ice sheets larger than today may be a critical ingredient for abrupt variations in NADW and climate. The concept of deep circulation control of North Atlantic heat flux provides a mechanism for explaining climate oscillations such as the Ognon I and II and Montaigu pollen events within the North Atlantic region, but the appearance of possibly correlative changes at more distant locations such as Antarctica and western North America probably requires additional feedbacks such as changes in water vapour content of the atmosphere³². □

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High-resolution climate records from the North Atlantic during the last interglacial

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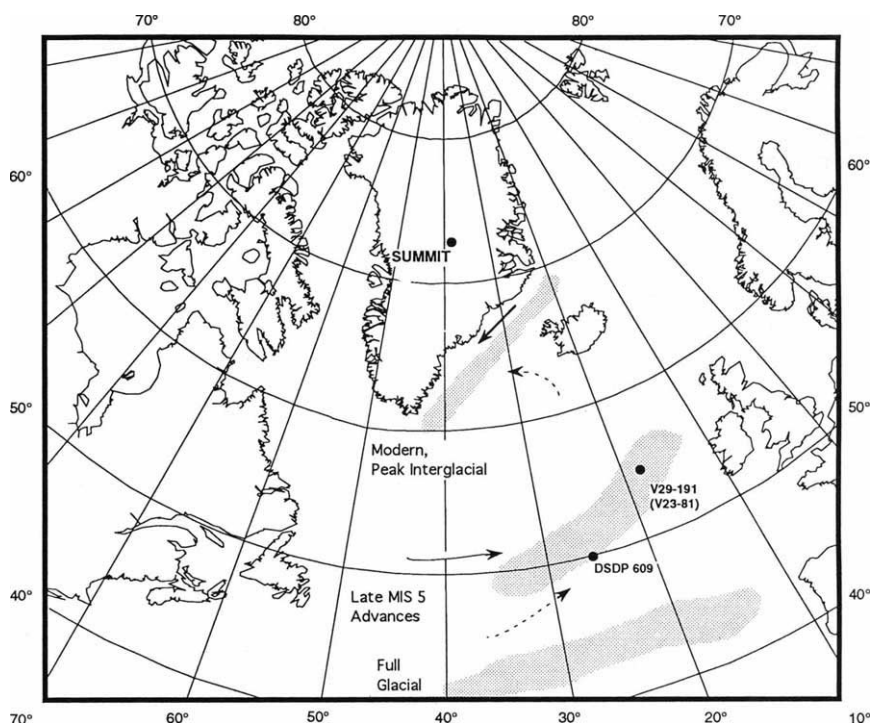
THE two deep ice cores recovered by the GRIP¹ and GISP² projects at Summit, Greenland, agree in detail over the past 100,000 years³ and demonstrate dramatic climate variability in the North Atlantic region during the last glacial, before the current period of Holocene stability. This glacial climate instability has subsequently been documented in the marine sedimentary record of surface-ocean conditions in the North Atlantic⁴. Before 100 kyr ago the two ice core records are discrepant, however, casting doubt on whether the oxygen isotope fluctuations during the last interglacial (Eemian) seen in the GRIP core^{1,5} represent a true climate signal.

Here we present high-resolution records of foraminiferal assemblages and ice-rafted detritus from two North Atlantic cores for the interval 65 kyr to 135 kyr ago, extending the surface-ocean record back to the Eemian. The correlation between our records and the Greenland ice-core records is good throughout the period in which the two ice cores agree, suggesting a regionally coherent climate response. During the Eemian, our marine records show a more stable climate than that implied by the GRIP ice core, suggesting that localized phenomena may be responsible for the variability in the latter record during the Eemian.

We have examined two cores (DSDP site 609, V29-191) from the eastern North Atlantic (Fig. 1) at a depth spacing corresponding to approximately 200 years throughout marine isotope stage 5 (MIS 5). This stage was broadly defined as an interglacial period in the original oxygen isotope stratigraphy⁶ and subsequently partitioned into substages 5a to 5e, with 5e alone correlative with the last interglacial (Eemian) in terrestrial records⁷. The two cores bear evidence of repeated encroachment of polar surface waters. While advances across a 20° swath of latitude have previously been documented on orbital timescales^{8,9}, water-mass migrations evident in this study are correlative with the higher frequency changes occurring on the Greenland ice sheet, beginning ~110,000 years ago^{1,2}. Since at least this time, cooling on the ice cap and at the sea surface have been linked.

Two indicators of oceanographic conditions were utilized for this study: the planktonic foraminiferal assemblage and the abundance of ice-rafted detritus (IRD), measured relative to coarse biogenic particles and to total sediment. Time series of these proxies are remarkably similar (Fig. 2). This similarity of

FIG. 1 Study area in North Atlantic. The Greenland location is the ice-core drilling site at the summit of the ice sheet. Ocean locations are sites of sediment cores used in this study. DSDP site 609 was drilled in a local basin on the eastern flank of the Mid-Atlantic Ridge. V29-191 was cored on an abyssal drift deposit along Feni ridge. V23-81 is a nearby core used for a previous comparison with the glacial portion of the Greenland record. Shaded areas represent the approximate location of polar water boundaries. Solid arrows indicate cold, fresh Arctic/sub-Arctic flows. Dashed arrows indicate warm, saline subtropical/subpolar flows.



sedimentary data from two widely spaced cores, representing the disparate depositional environments of local basin and abyssal drift, attests to the accurate recording of widespread oceanographic conditions.

Each of our records contains peaks of polar foraminifera (*N. pachyderma* s.) and IRD which rise above low ambient values. This background has a value of a few per cent for much of the study interval, and is at or near zero in sediment representing MIS 5e. Eight warm-cold oscillations are evident in the faunal record between the glacial inception at ~110 kyr (before the present) and the end of MIS 4. The ramping structure so prevalent in the subsequent glacial period⁴ is absent here. IRD abundances increase and diminish repeatedly, culminating in Heinrich event H6 (ref. 10).

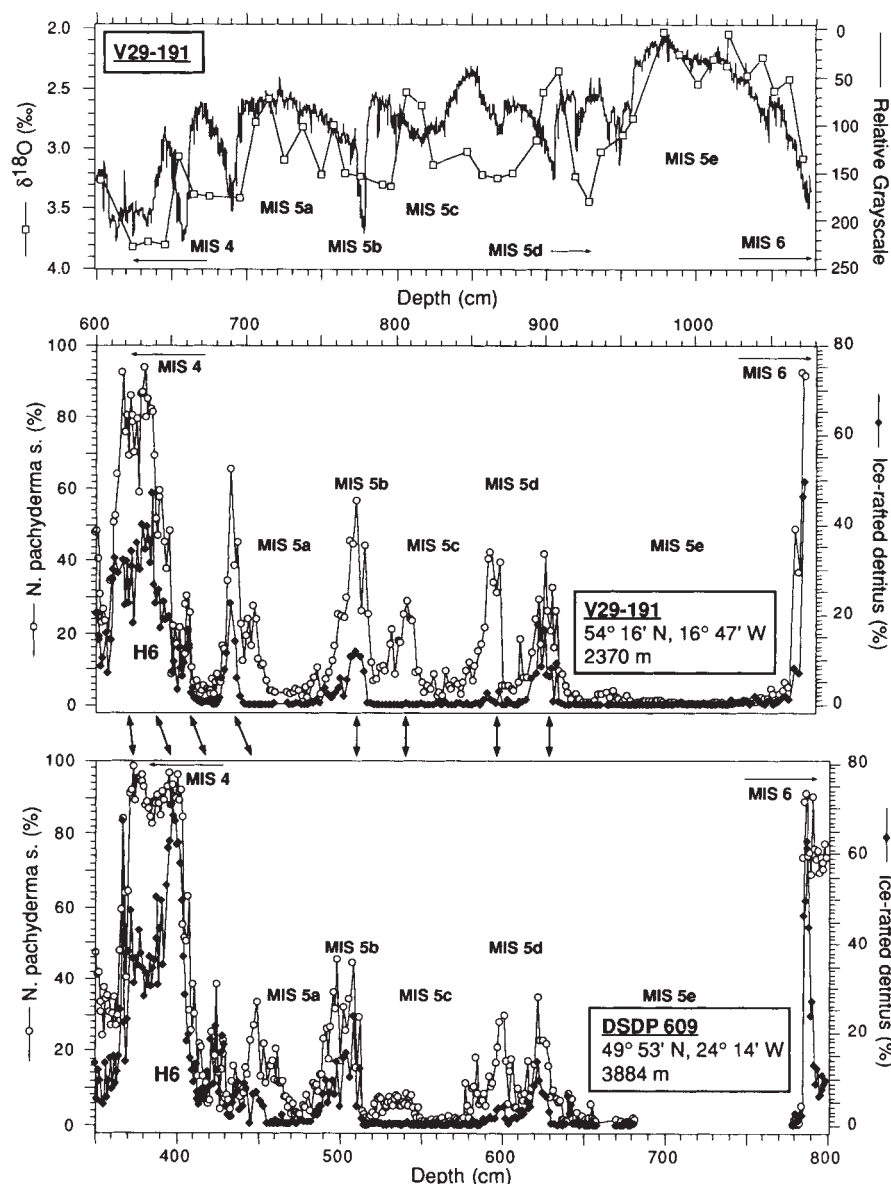
Relatively mild conditions prevailed in the subpolar surface North Atlantic for most of MIS 5, while polar conditions occurred as transients. This finding is consistent with the view that maximum warmth is limited to the peak interglacial MIS 5e while recurrent climate amelioration occurred throughout MIS 5 (ref. 11). Similar evidence of mild climate punctuated by cool

intervals characterizes high-resolution terrestrial records which are interpreted as correlative with MIS 5 (refs 12, 13).

Episodic polar influence at these sites implies the repositioning of an oceanic front at the boundary between surface currents originating in the Arctic and subtropical Atlantic (Fig. 1). On a glacial/interglacial timescale, frontal migrations over a given site are captured in sediments as a nearly square-wave transition between extreme proxy values. The moderate abundances of polar fauna during cool events within MIS 5 indicate the proximity of our core locations to a dynamic polar front. As indicated by the peak values, V29-191 was near the mean position of the frontal zone during intermediate polar advances, while DSDP site 609 was nearer the southern edge of the zone.

The inferred location of the surface water-mass boundary has important implications for thermohaline circulation. Such a configuration implies restricted northward export of saline surface waters from the Atlantic in conjunction with reduced deep ventilation and production of North Atlantic Deep Water (NADW) in the Nordic Seas. Deep circulation is one mechanism capable of influencing Greenland air temperatures, by way of surface

FIG. 2 Time series showing climate proxies from marine sediment cores plotted against depth. Oxygen isotopes were obtained from the benthic foraminifera *Cibicidoides*. Greyscale curve is derived from relative reflectivity measurements on digitized sediment images. *Neogloboquadrina pachyderma sinistral*, a planktonic foraminifera, is associated with polar surface waters. Variations in the abundance of this species, measured as a percentage of the total planktonic foraminifera, are indicative of the prevalence of polar conditions at a given site during the several centuries represented by a single sample. Arrows indicate eight peak polar abundances. Increases in polar foraminifera come largely at the expense of a subpolar assemblage dominated by *Neogloboquadrina pachyderma dextral* and *Globigerina bulloides*. Ice-rafted detritus abundance is calculated as the percentage of coarse terrigenous grains (>150 µm) to total coarse particles including biogenic remains. Such large particles are unlikely to be carried long distances by mechanisms other than floating ice^{23,24}.



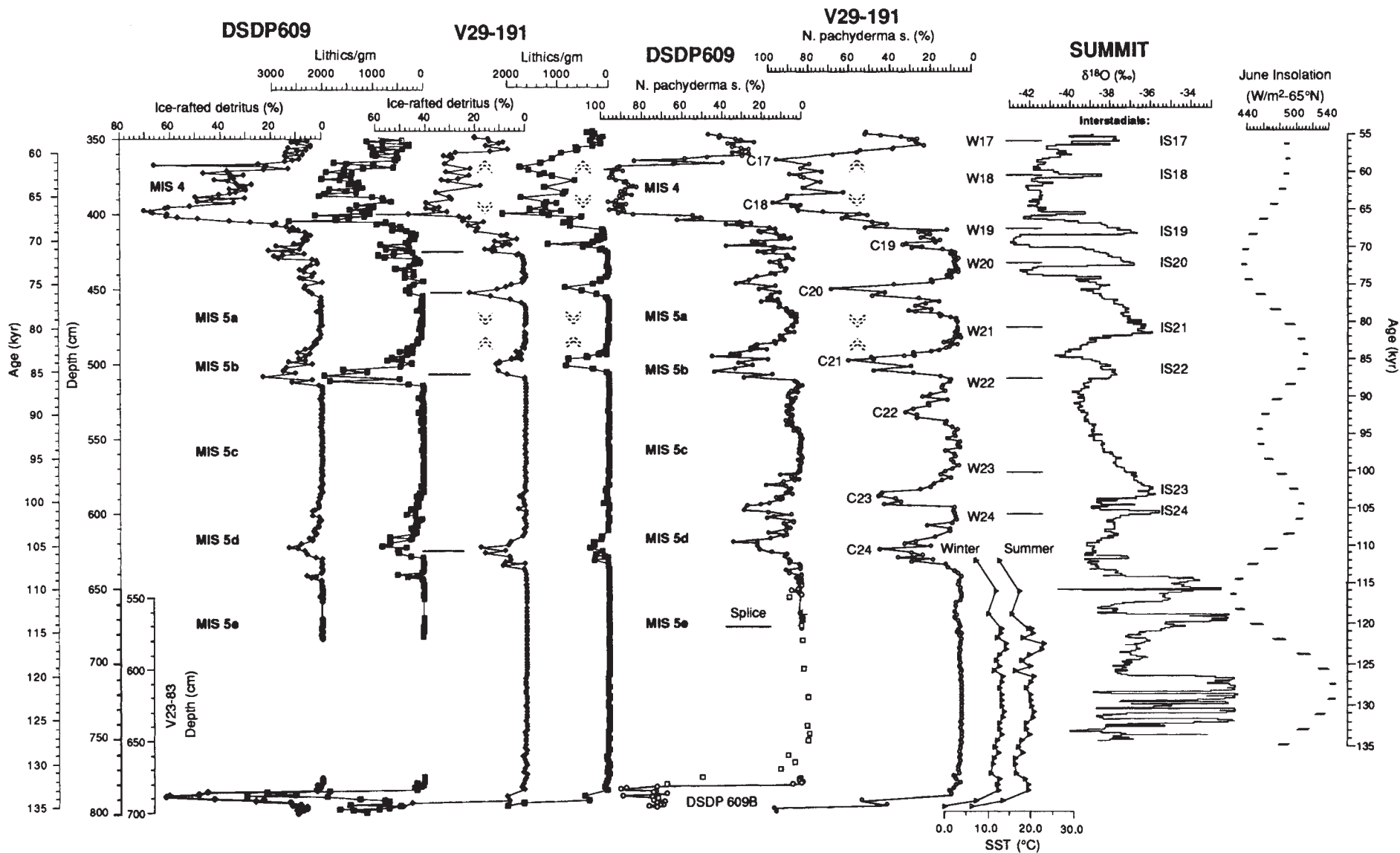


FIG. 3 Comparison of deep sea cores with GRIP ice core from Summit, Greenland. Isotopic composition of precipitation over Greenland, preserved as ice, reflects distillation of atmospheric water vapour during poleward transport. Ice core is plotted against age based on flow of thinning ice. DSDP site 609 is plotted against depth below the sea floor. Spliced data is from nearby core V23-83 (ref. 25): V29-191 record has been stretched in MIS 4 and compressed in MIS 5a to match

DSDP site 609, accounting for differences in sedimentation rates. Numbered marine polar episodes, C; Numbered subsequent warmings, W. Lithic concentrations and IRD per cent represent the ratio of coarse ($>150 \mu m$) terrigenous fragments to bulk sediment weight and total coarse particles (terrigenous fragments + foraminifera tests) respectively.

ocean heat transport, on the observed millennial timescales, which are not easily explained by orbitally influenced insolation anomalies. Changes in the mode or flux of NADW would also provide global communication of regional conditions.

The relationship between IRD and the foraminiferal assemblage is interesting. Each increase in IRD is associated with an increase in polar foraminifera but not all the increases in polar foraminifera are accompanied by increased IRD. This distinction is important. Colder seas occurred in conjunction with the arrival of debris-laden icebergs, but colder seas alone were insufficient to enhance IRD. The IRD influxes are equal in number and similar in amplitude to increases in $\delta^{18}\text{O}$ apparent in high-resolution benthic records¹⁴, and may be linked to early stages of ice growth on surrounding land masses. Greater amounts of IRD at DSDP site 609 than at V29-191 thus reflect iceberg surface trajectory, as proposed for younger IRD events¹⁵, and enhanced deposition at the southern edge of an iceberg-laden current.

Enhanced IRD deposition began with the initial advance of polar waters relatively early in MIS 5. This may indicate rapid growth of Northern Hemisphere glaciers, accounting for much of the fall in sea level and increase in seawater- $\delta^{18}\text{O}$ associated with MIS 5d. Alternatively, nucleation of glaciers may have occurred near enough to the surrounding coasts to allow ice margins to reach the sea early during ice growth.

It is interesting to consider the IRD peaks in the context of the six Heinrich events of the last glacial period¹⁵. Our study reveals four more events within MIS 5 and another at termination 2, for a total of 11 during the last climate cycle. This is exactly the number postulated by Heinrich¹⁶, but not identified in his study of low sedimentation-rate cores. The event associated with termination 2 shares a suite of characteristics with the younger glacial events¹⁷ (for example, detrital carbonate, foraminifera minimum), while it remains to be seen if the MIS 5 events constitute interglacial equivalents of Heinrich events.

The eight intervals of enhanced polar influence are easily correlated with decreased temperature on Greenland, as deduced from ice sheet $\delta^{18}\text{O}$. Subsequent retreats of polar waters thus correspond to the oldest eight interstadials identified in Greenland ice (Interstadials 17–24)¹. Each of the warm and cold events can be matched between records on a one-to-one basis (Fig. 3), as well as on the basis of pairs of cold events associated with MIS 4, 5b, 5d and the 4–5 boundary. This observation supports a climatic interpretation of this portion of the Greenland record and is further evidence of the deep sea's capacity to record high-frequency variability.

We used published age models for the GRIP ice core¹ and DSDP site 609 (ref. 4) to establish a chronology for comparison. Although temporal models based on the flow of thinning ice and constant sediment accumulation contain significant uncertainties, these independent timescales put our correlation to a simple test which goes beyond establishing that eight events occurred during a given interval. The largest difference between age esti-

mates for correlative cold events occurs for the earliest two, and are of the order of 5,000 yr (Fig. 3). All of the others agree with an average of 1,200 yr, a reasonable figure given the estimated errors and uncertain phase relationships.

Differences in the amplitudes of excursions in the compared records are evident in two cycles on the MIS 4–5 transition. They are anomalously pronounced in the ice cores, to the extent that their isotopic composition implies conditions which were not only colder than the ensuing MIS 4 but more severe than virtually all of peak glacial MIS 2. Our records of the MIS 4–5 transition more closely resemble the distinct intermediate oscillations previously documented in sea surface¹⁸ and deep ocean records¹⁹. These events are real, yet their ice core expression may incorporate differing water-vapour sources²⁰ and/or may reflect local or high latitude climate amplification, perhaps associated with the glacial transition.

Below substage 5d, the correspondence between our marine records and the ice core record breaks down. This observation bears reflection, considering the subsequent similarity. Because DSDP site 609 is not continuous through MIS 5e, V29-191 is better suited for comparison. The signature, if any, of unstable peak interglacial climate in this core is extremely muted, in contrast to the clear expression of younger events that are similar in amplitude and duration. One possibility for this lack is that our proxies are saturated throughout and that the Eemian climate was unstable while remaining warmer than all ensuing intervals of MIS 5. While this view is not completely supported by the GRIP record itself, which implies Eemian intervals substantially cooler than subsequent interstadials and nearly as cold as some subsequent stadials, it does appear that the abundance of polar fauna reaches a practical minimum in MIS 5e. Estimates of sea surface temperatures derived from the more diverse foraminiferal assemblage display a limited amount of variability about a longer term rise and fall through the peak interglaciation. A second possibility is that the subpolar North Atlantic was decoupled from atmospheric conditions over Greenland during MIS 5e²¹. In this case the GRIP record from Summit may contain the imprint of local climate variability. A third possibility is that the Eemian instability represents a different non-local phenomenon from the ensuing record, for example source region variability rather than temperature. It is also possible that the integrity of the ice core records has been compromised. Duplicate records remain the best test of this possibility, and they are most likely to come from the deep sea. Recent evidence from the Bahama Outer Ridge suggests a similar structure in benthic indicators²². No equivalent ice cores will be recovered for some time, and while careful scrutiny has failed to demonstrate anomalous deformation within the mid and late Eemian section of the GRIP summit core, the GISP2 ice core, drilled near the summit site, is clearly disturbed throughout the interval of interest³ and cannot be used to support the GRIP record. Although this is not the last word on Eemian instability, the deep-sea record appears to hold the key in the near future. □

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Ecological and temporal placement of early Pliocene hominids at Aramis, Ethiopia

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SEDIMENTARY deposits in the Middle Awash research area of Ethiopia's Afar depression have yielded vertebrate fossils including the most ancient hominids known. Radioisotopic dating, geochemical analysis of interbedded volcanic ashes and biochronological considerations place the hominid-bearing deposits at around 4.4 million years of age. Sedimentological, botanical and faunal evidence suggests a wooded habitat for the Aramis hominids.

The Middle Awash research area straddles the modern Awash River south of Hadar (Fig. 1). Geological work began in 1938¹ and Taieb discovered the area's palaeoanthropological significance in the 1960s². Research was extended by the RVRME (Rift Valley Research Mission in Ethiopia) between 1975 and 1978^{3,4} and by our team beginning in 1981^{5,6}. Stratigraphic inter-

pretation is hindered by complex syn- and postdepositional faulting and uplift, rapid facies changes, repetitive depositional regimes and erosion. But tephrochemical and radioisotopic dating of numerous volcanic horizons, together with biochronological analyses of abundant fossils, have revised and extended previous interpretations.

The earliest deposits investigated crop out along the western Afar margin. These are biochronologically late Miocene in age (the RVRME 'Adu' and 'Asa' Members). No hominid fossils have been recovered from them. The thickest and most widely exposed Middle Awash sediments are Pliocene, cropping out east and west of the modern river. The top of the mostly lacustrine VT-1 (=Moiti Tuff 3.89 ± 0.02 Myr⁶) through SHT (Sidi Hakoma Tuff; 3.40 ± 0.03 Myr⁶) succession exposed east of the modern Awash correlates with the lower part of the Hadar Formation to the north (errors quoted throughout at one standard deviation)^{6,7} (Fig. 1). These sediments yielded *Australopithecus afarensis* dated to ~3.4 and 3.8 Myr at Maka and Belohdelie^{6,8}. Uplifted Pliocene sediments west of the Awash (the 'Central Awash Complex'⁹) extend this record deeper in time. Recent fieldwork has established the sub-Cindery Tuff (CT; 3.85 ± 0.03 ; ref. 5) succession, including the CT and VT-1, west of the modern river.

Following Gen Suwa's discovery of hominid fossils in the Aramis headwaters on December 17, 1992 (see ref. 10) investigation focused on the section exposed between the upper Adgantoli and lower Sagantole drainages (Fig. 1). Here, progressively older sediments were uplifted above the Awash valley floor and are now exposed on the flanks of the central complex. These beds, the RVRME's 'Aramis' and 'Haradaso' Members, were placed biochronologically between 3.5 and 4.5 Myr (ref. 11).

We found the VT-1/Moiti tuff near Mee-ee Koma, east-south-east of the Aramis area (Fig. 1). We have shown elsewhere that the VT-1/Moiti (3.89 ± 0.02 Myr) is not the earliest volcanic unit in the Middle Awash⁷ (cf. refs 11 and 12). Studies are underway to determine the total thickness of the sub-VT-1/Moiti sedi-

TABLE 1 Chemical composition of Aramis volcanic glasses

Sample	Location	n	SiO ₂	TiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MnO	MgO	CaO	Na ₂ O	K ₂ O	Total
Gàala tuff complex (GATC)												
Endmember silicic glass population												
MA92-12A	SAG	26	73.78	0.15	11.00	1.87	0.03	<DL	0.29	2.36	3.54	93.01
MA92-12B	SAG	27	72.46	0.13	11.06	1.87	0.02	<DL	0.29	2.46	4.00	92.29
MA92-12C	SAG	5	71.83	0.15	11.14	1.92	0.04	<DL	0.32	2.29	3.55	91.23
MA92-37A	SAG	32	72.35	0.14	11.41	1.87	0.03	<DL	0.32	2.53	5.01	93.67
MA92-37B	SAG	18	72.16	0.13	11.37	1.94	0.04	<DL	0.30	2.47	4.91	93.32
MA93-24	ARA	28	71.59	0.13	11.38	1.95	0.03	<DL	0.31	2.26	4.49	92.15
MA93-37	ARA	37	72.37	0.13	11.24	1.89	0.03	<DL	0.29	2.50	4.52	92.97
MA93-39	ARA	2	72.27	0.12	11.22	1.80	0.03	<DL	0.28	2.75	4.89	93.36
Endmember mafic glass population												
MA92-12C	SAG	10	54.14	2.40	13.53	13.56	0.23	2.73	6.05	2.96	2.06	97.65
MA92-37B	SAG	5	54.81	2.33	13.55	13.46	0.23	2.78	6.08	2.83	2.09	98.16
MA93-39	ARA	5	55.21	2.32	13.56	13.19	0.23	2.83	5.94	3.09	2.05	98.42
Daam Aatu basaltic tuff (DABT)												
MA92-38B	ARA	28	49.81	3.31	12.70	15.66	0.21	4.43	8.42	2.18	0.78	97.51
MA92-39	ARA	14	50.62	3.38	12.76	15.73	0.20	4.72	8.44	2.30	0.71	98.87
MA93-25	SAG	27	50.74	3.27	12.80	15.41	0.20	4.48	8.08	2.34	0.75	98.06
MA93-42	ARA	21	51.68	3.30	12.90	15.20	0.20	4.52	8.09	2.19	0.75	98.82

Major element oxides in weight per cent with total Fe expressed as Fe₂O₃. Both silicic and basaltic glass shards were analysed at Los Alamos National Laboratory by an automated SX50 Cameca electron microprobe operated using an accelerating potential of 15 kV, 15 nA beam current and a fixed beam size of 10 µm. Standards used for calibration include both natural silicic and basaltic glasses, a set of feldspars, iron oxide, pyroxenes and amphiboles. The tuff samples consist of Y-shaped, blocky to elongate thin or broken platy and sparsely-vesicular shards. Finely vesicular and elongate pumice clasts are common. Some of the silicic tuffs contain coarsely vesicular scoria clasts and brown vesicular droplets with occasional quench textures from mixed magma eruptions. Location indicates major drainage from which samples were recovered, with SAG = Sagantole and ARA = Aramis. The total number of shards used in the averages is represented by n. Spatial and stratigraphic placements for samples are shown in Fig. 1. DL, below detection limits.

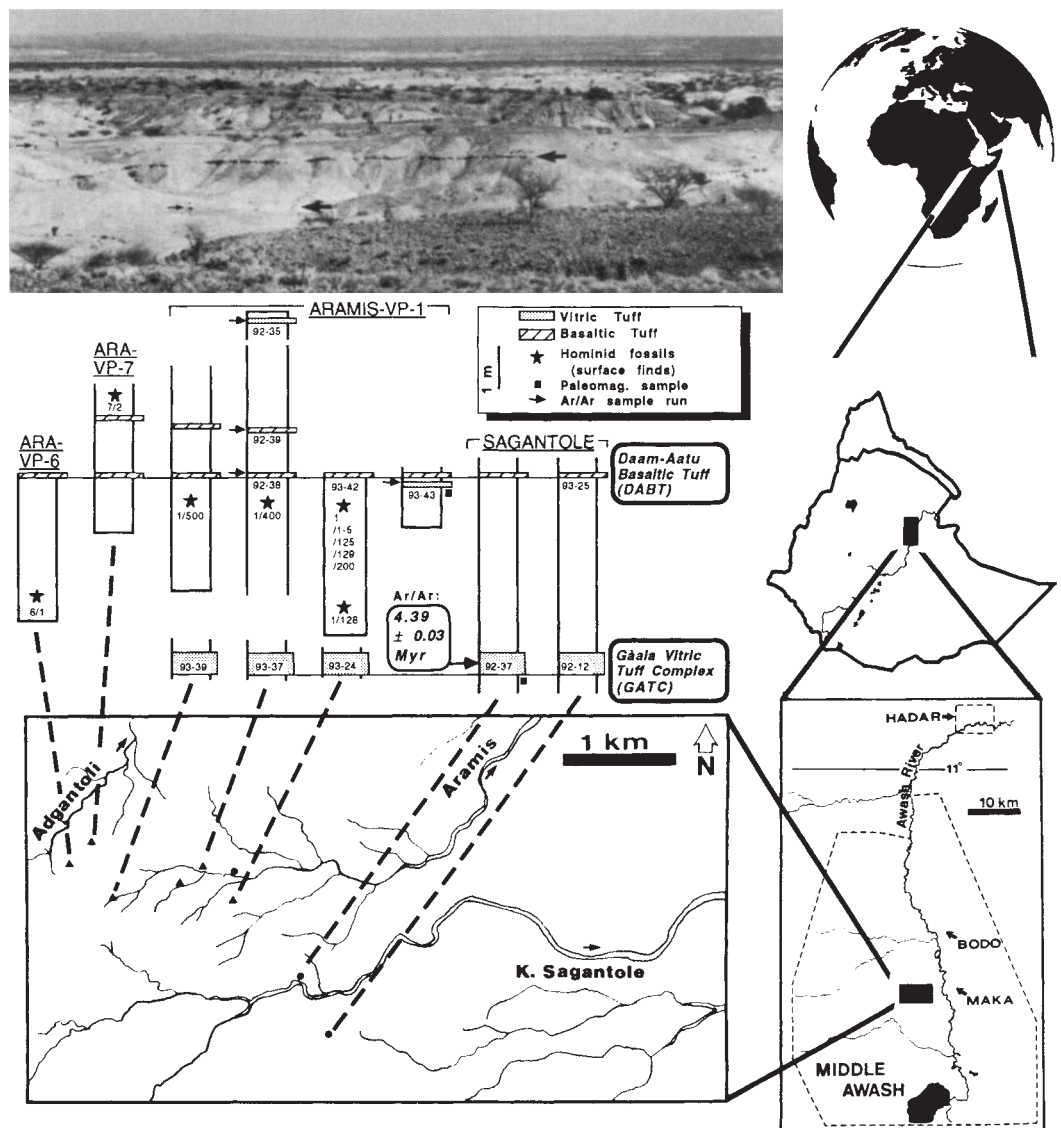
ments in the Aramis/Sagantole area. Pliocene strata here generally dip about 5° to the east, beneath the modern Awash floodplain. These deposits, including many volcanic strata, lie beneath the VT-1/Moiti tuff on structural, biochronological, radioisotopic and geochemical grounds.

Sediments that geographically span the Aramis/Sagantole/Adgantoli regions include a prominent, diagnostic vitric tuff complex named here the Gāala Tuff Complex (GATC, 'gāala' is 'camel' in the Afar language; Fig. 1). Major features of the local section are traceable and recognizable over a distance of ~ 4 km from northwest to southeast. A prominent, evenly stratified, grey to black basaltic tuff, the Daam Aatu Basaltic Tuff (DABT, 'daam aatu' is 'monkey' in the Afar language) is found, on average, about 4 m above the GATC across the same distance. The vast majority of vertebrate remains discussed below are derived from between these marker beds. The GATC silicic and DABT basaltic tuff strata are distinct regional marker beds. Correlations among samples of these marker tuff units were established using field observations together with chemical compositions of glass separated from the silicic and basaltic tephra (Fig. 1 and Table 1). Many of the GATC silicic tuffs contain a

complex mixed glass population with identifiable high and low silica endmember modes. On the basis of scanning electron microscope observations, these mixed populations are considered primary features of the volcanic system, thus geochemical correlations are based on similarities between dominant glass populations.

We have attempted to date several of the tuff units interbedded with the hominid-bearing sediments (Fig. 1) by the single crystal laser fusion $^{40}\text{Ar}/^{39}\text{Ar}$ method. Most of the feldspar grains separated for these analyses are contaminated by a dominant population of sanidine grains yielding an early Miocene age (~ 23.5 Myr). Volcanic rocks represented in the escarpment and plateau to the west are likely sources of these contaminant feldspars. One sample of the GATC (MA92-37), although showing this early Miocene contamination, yielded a dominant feldspar population providing a mean age of 4.387 ± 0.031 (s.e.) Myr on the basis of 17 individual crystals (Table 2). This age is viewed as the best estimate for the age of the GATC, and thus provides a maximum age for the hominid remains. The remaining nine grains from this sample represent contaminants, primarily of a Miocene population dated to 23.6 ± 0.01 Myr. We encountered

FIG. 1 Placement map and stratigraphic diagrams. Photo is an overview of the Middle Awash study area. View is to the east, across the dark horizontal line of vegetation that marks the course of the modern Awash river. The Aramis catchment is to the left, Sagantole to the right. White outcrops beyond the river are ~ 3.8 Myr diatomites in the vicinity of Maka and Wee-ee. Human figures for scale are marked by small arrows. Beds dip synthetically, away from the viewer and toward the Awash. The dark horizontal band in the foreground (upper large arrow) is the Daam Aatu Basaltic Tuff (DABT). This dark-olive tuff is coarse-grained with abundant basaltic glass and rock fragments. Its distinctive bedded and laminated lower part ranges in thickness from about 20 to 40 cm. The white tuff below is the Gāala Vitric Tuff Complex (GATC; lower large arrow), dated here to 4.387 ± 0.013 Myr. This white to grey vitric tuff is more than 1 m thick and grades upwards to a coarser and pumiceous zone. This unit predates the prominent marker tuffs from: (1) the Deep Sea Drilling Project (DSDP) cores from the Gulf of Aden and the Indian Ocean; (2) Hadar and Maka/Belohdelie of the Middle Awash; and (3) the Omo and Turkana Basins in southwest Ethiopia and northern Kenya. All but one hominid specimen come from deposits sandwiched between these tephra. Numbers beneath the stars



indicate important hominid specimens. Numbers beneath the tephra are prefaced by the MA (Middle Awash) designation and indicate year and sample number.

TABLE 2 $^{40}\text{Ar}/^{39}\text{Ar}$ analytical data for MA92-37

Lab no.	^{40}Ar (mol) ($\times 10^{-15}$)	$^{40}\text{Ar}/^{39}\text{Ar}$	$^{37}\text{Ar}/^{39}\text{Ar}$	$^{36}\text{Ar}/^{39}\text{Ar}$ ($\times 10^{-4}$)	$^{40}\text{Ar}^*/^{39}\text{Ar}$	% $^{40}\text{Ar}^*$	Age (Myr)	± 1 s.d. (Myr)
7198-01	29.701	28.040	0.006	1.580	27.988	99.8	23.763	0.067
7198-02	135.071	28.356	0.005	4.026	28.232	99.6	23.969	0.039
7198-03	7.641	9.705	0.000	0.998	9.670	99.7	8.246	0.058
7198-04	2.142	5.097	0.016	0.409	5.081	99.8	4.337	0.102
7198-05	4.528	5.072	0.082	0.393	5.062	99.9	4.321	0.049
7198-06	4.465	5.101	0.014	0.179	5.092	99.9	4.347	0.030
7198-07	5.667	5.106	0.025	0.371	5.092	99.8	4.347	0.024
7198-08	36.442	29.452	0.003	45.598	28.100	95.4	23.857	0.058
7198-10	16.892	27.786	0.034	0.254	27.776	100.0	23.584	0.086
7198-11	2.472	5.256	0.019	1.477	5.208	99.2	4.446	0.053
7198-12	28.020	27.995	0.000	6.321	27.804	99.3	23.607	0.053
7198-13	0.494	7.013	12.418	37.154	6.904	97.8	5.891	0.492
7198-14	0.161	8.752	12.306	141.271	5.547	63.0	4.735	1.283
7198-16	2.093	5.328	0.263	3.974	5.226	98.2	4.461	0.087
7198-17	1.537	5.457	0.013	5.694	5.284	96.9	4.511	0.138
7198-18	2.808	5.155	0.025	2.685	5.072	98.5	4.330	0.047
7198-19	0.716	5.234	0.015	4.677	5.092	97.4	4.346	0.166
7198-20	1.642	5.292	0.014	5.188	5.135	97.1	4.383	0.076
7198-21	11.684	39.975	0.219	65.437	38.058	95.2	32.236	0.129
7198-22	1.448	5.387	0.302	13.071	5.019	93.2	4.285	0.089
7198-23	2.437	5.191	0.023	4.365	5.058	97.5	4.318	0.053
7198-24	0.238	6.591	1.680	63.721	4.835	73.3	4.128	0.607
7198-25	0.496	5.821	0.031	19.542	5.240	90.1	4.473	0.231
7198-26	0.394	5.506	0.087	9.423	5.229	95.1	4.464	0.332
7198-27	3.447	5.202	0.031	3.631	5.092	98.0	4.347	0.042
7198-28	8.171	30.899	0.000	106.765	27.739	89.8	23.552	0.151

$^{40}\text{Ar}/^{39}\text{Ar}$ analytical data are from individual 300 to 700 μm feldspar crystals from sample MA92-37 dated using the facilities and procedures described previously¹⁷. Relative isotopic abundances are corrected for mass discrimination, radioactive decay and procedural blanks. Blank values were similar to those in ref. 17. Samples were irradiated at the Oregon State University TRIGA reactor. Neutron fluence parameter $J = 4.737 \times 10^{-4} \pm 3.0 \times 10^{-6}$, determined by individual analysis of seven co-irradiated grains of Fish Canyon sanidine (27.84 Myr). Samples were shielded by Cd to minimize the thermal neutron fluence. Interfering isotope corrections were determined from analysis of co-irradiated CaF_2 and synthetic KFeSiO_4 glass, which yielded the following nucleogenic production ratios: $(^{39}\text{Ar}/^{37}\text{Ar})_{\text{Ca}} = 0.00066 \pm 0.00024$, $(^{36}\text{Ar}/^{37}\text{Ar})_{\text{Ca}} = 0.00026 \pm 0.00001$, $(^{40}\text{Ar}/^{39}\text{Ar})_{\text{K}} = 0.0051 \pm 0.0025$. The mean age and s.e. of 17 grains shown in boldface is 4.387 ± 0.031 Myr.

pervasive Miocene contamination in the DABT and the overlying MA93-35 and underlying MA93-43 samples (Fig. 1). No Pliocene feldspars were found in these overlying strata. A total of 41 grains encompassing the full range of colour, size and morphology were dated, and the combined results from these three strata are indistinguishable from the Miocene feldspar contaminant grains dated in the MA92-37 sample. Samples for palaeomagnetic analysis were taken from immediately below the GATC and DABT horizons (Fig. 1). The first yielded a steep characteristic direction of uncertain polarity, whereas the second (DABT) was unambiguously reversed. This finding, in combination with the $^{40}\text{Ar}/^{39}\text{Ar}$ and biochronological data, suggests that these sediments were deposited between the top of the Ninivak subchron (4.48 Myr) and the bottom of the Cochiti subchron (4.29 Myr)¹³.

The dated Gåala (GATC; 4.387 ± 0.031 Myr) and undated Daam Aatu volcanic strata sandwich all but one of the hominid specimens. This is a left arm found ~0.6 m above the DABT in Aramis Locality 7 (Fig. 1). Stratigraphically contemporaneous fossils (from within 3 m above the DABT at Locality 7 and elsewhere) comprise less ravaged assemblages with more complete specimens, including crania and articulated elements, and suids and elephants that are not biochronologically different from those found below the DABT. Further sampling of these more fluviatile beds above the DABT is required to assess the fauna found with the associated hominid arm elements at Locality 7.

The remainder of the hominid fossils and all of the vertebrate fauna analysed below derive from 1 to 3 m of characteristically loose stratified silt with development of red 'mediterranean' soils (hue 5YR). The primary fossil vertebrate horizon rests atop a prominent, fairly continuous carbonate unit containing abundant fossil seeds and wood, as well as some insect pupal cases and spirostreptid millipede remains. This weakly cemented unit varies up to 0.5 m in thickness and is usually found 0.5–1.5 m

below the basal contact of the DABT. The primary fossiliferous horizon was deposited in the course of a stabilizing pediment.

After the discovery of hominids in the centre of Aramis Locality 1 (ARA-VP-1; $10^\circ 28' 35.8''$ N, $49^\circ 27' 11.6''$ E, elevation ~600 m), an intensive effort was devoted to recovering additional fossils here and in adjacent localities. This resulted in recovery of 649 identifiable vertebrate specimens to date, including 16 certain hominid specimens from between the GATC and DABT¹. Table 3 provides a list of vertebrate taxa found between these volcanic strata at Aramis. Figure 1 shows the spatial dispersion of these finds. The medium and large mammal bones were fragmented before fossilization. Isolated teeth dominate the combined assemblage. Limb bone shaft fragments are numerous, generally unweathered, and unabraded by fluvial action or stomach acids. Intact bones are virtually nonexistent except for small bones of the hands and feet. Articular ends are usually missing. Carnivore tooth marks scar the hominid cranial and postcranial elements and are ubiquitous on medium and large mammal bones in general. A full range of mammal size is represented, from bats to elephants. To date, the hominid remains do not cluster spatially, but follow a scattered distribution similar to that seen for the other mammals such as carnivores, bovids and colobine monkeys collected from the same stratigraphic interval across linear kilometres of outcrop (Fig. 1). We interpret the physiographical setting to have been a flat plain with little topography where scattered carcasses of medium and large mammals were ravaged by carnivores but not concentrated across the broad area sampled. There is no sedimentological or bone modification evidence of fluviatile transport. There is no difference between the hominids and other medium-sized mammals in spatial distribution, bone modification or element representation.

The carbonate horizon forming the base of the primary fossil vertebrate horizon is recognizable across 3 km of outcrop. Fossilized plant remains in this stratum are ubiquitous, and include thousands of *Canthium* seeds, a genus common in

TABLE 3 Faunal list for Aramis vertebrate fossil localities

Aves	Primates
Two sizes	Colobinae
Reptilia	cf. <i>Paracolobus</i> sp.
Chelonia	cf. sp. "A"
Lacertilia	cf. <i>Parapapio</i> sp.
Crocodylia	Proboscidea
cf. <i>Python</i>	Elephantidae sp.
Rodentia	<i>Anancus</i> sp.
<i>Tachyoryctes</i> sp.	<i>Deinotherium</i> sp.
<i>Golunda</i> sp.	Bovidae
<i>Millardia</i> sp. aff. <i>taiebi</i>	<i>Tragelaphus</i> sp.
<i>Millardia</i> sp. aff. <i>coppensi</i>	Bovini sp.
Sciuridae	Neotragini sp.
Chiroptera	Perissodactyla
Microchiroptera	<i>Ceratotherium</i> cf. <i>praecox</i>
Carnivora	<i>Hipparion</i> sp.
Hyaenidae	Suidae
Mustelidae	<i>Nyanzachoerus jaegeri</i>
<i>Torolutra</i> aff. <i>ougandanensis</i>	<i>Nyanzachoerus kanamensis</i>
Lutrinae indet.	Giraffidae
Viverridae	<i>Giraffa</i> aff. <i>stillei</i>
Canidae	<i>Giraffa</i> aff. <i>jumae</i>
cf. aff. <i>Nycterentes</i> sp.	Hippopotamidae
Felidae	<i>Hexaprotodon</i> sp.
cf. <i>Megantereon</i> sp.	
Ursidae	
<i>Agriotherium</i> sp.	
<i>Helogale</i> aff. <i>kitafe</i>	

Faunal list for Aramis vertebrate fossil localities stratigraphically placed between the DABT and GATC. These taxa are stratigraphically and usually spatially associated with the fossil hominid remains.

African woodlands and forests. Microfauna is abundant in the sub-DABT unit. Complete rodent skulls and isolated mandibles were recovered without washing during hominid surface collection. The micromammalian fauna includes a bat, a small squirrel, the dwarf mongoose *Helogale*, the bush rats *Golunda* *Millardia* and the mole rat *Tachyoryctes*.

The large mammal assemblage taxonomic composition and relative abundance values are unique among Plio-Pleistocene hominid occurrences. Aquatic elements (turtle, fish, crocodile) are rare. Large mammals (hippopotamus, proboscideans, rhinos, equids, giraffids, bovines) are rare. Primates are very abundant, comprising a large percentage of the overall fauna. There are at least three primate taxa. These comprise the hominids, at least one medium-sized colobine monkey and a papionine. The colobines are most abundant, accounting for over 30% of all collected vertebrate specimens (all genus-identifiable mammalian fossils found between the GATC and DABT were collected). Carnivores are diverse and include the first eastern African occurrence of the ursid *Agriotherium* as well as otters, hyaenids, viverrids and felids. The bovid fauna is dominated by abundant remains of a medium-sized kudu, *Tragelaphus* sp. Remains of other bovid taxa are extremely rare. They span the size range from gazelle-sized neotragines to large bovines. The dominance of colobine monkeys and kudus is a strong indication of a closed, wooded Pliocene Aramis environment. The *Tachyoryctes* and nearby Hadar pollen data suggest that Pliocene deposition at Aramis took place at least 1,000 m higher than the modern elevation, with subsequent downfaulting after 2.9 Myr (ref. 14).

The remains of 16 hominid individuals were discovered at Aramis in these taphonomic circumstances. They were distributed widely and accompanied by the flora, fauna and over-printed palaeosol described above. We interpret these initial results as evidence that the hominids lived and died in a wooded setting. The hominid fossil record is very poor beyond 4 Myr ago. Part of this scarcity may be attributable to discovery bias because relatively few sites sample this time period. However, the spatial, stratigraphic, faunal and floral associations of the

Aramis hominids may shed additional light on the paucity of hominid fossils in other African deposits of equal and greater antiquity. The distinctive high colobine monkey content of the associated fauna, and the rarity of equids, proboscideans and non-tragelaphine bovids is striking in comparison with other hominid-bearing deposits in the Middle Awash and elsewhere^{15,16}. *A. afarensis*, the species presumably descended from the Aramis hominids, is inferred to have displayed wide ecological tolerances—its remains are known from relatively open habitats. It is possible that the earlier Aramis hominids avoided these more open environments and predated the expansion out of wooded habitats recorded for *A. afarensis*. This could explain the scarcity of basal Pliocene hominid remains in non-woodland settings in the Middle Awash and beyond. □

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Essential role of *Mash-2* in extraembryonic development

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THE outer layer of the blastocyst, or trophoblast, is the first cell lineage to differentiate in the mouse embryo^{1,2}, but little is known about the genetic control of its development. Lineage-specific transcription factors may be important in lineage specification, and the product of the *Mash-2* gene^{3,4} fulfils the criteria for such a factor. *Mash-2* is a mammalian member of the *achaete-scute* family^{5–7} which encodes basic-helix-loop-helix transcription factors⁸ and is strongly expressed in the extraembryonic trophoblast lineage. *Mash-2* transcripts are found in the female germ line

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and in the embryo throughout preimplantation development, but are highly expressed later only in the ectoplacental cone, the chorion and their derivatives in the placenta. *Mash-2* transcripts are not found in primary and secondary giant cells, yolk sac or allantois at any post-implantation stage, and are present only transiently and at low levels in the embryo during gastrulation. To analyse the role of *Mash-2* in development, we have used gene targeting to generate mice having no *Mash-2* function. We report here that *Mash-2*^{-/-} embryos die from placental failure at 10 days post-coitum. In mutant placentas, spongiotrophoblast cells and their precursors are absent and chorionic ectoderm is reduced. We have rescued this placental mutant phenotype by constructing chimaeras with tetraploid wild-type embryos which contribute almost exclusively to extraembryonic tissues^{9,10}. *Mash-2*^{-/-} embryos developed normally and adult *Mash-2*^{-/-} mice were viable, demonstrating that *Mash-2* has no major role in the embryo itself. *Mash-2* is the first transcription factor shown to play a critical part in the development of the mammalian trophoblast lineage.

A mutation in *Mash-2* was created by replacing most of its amino-acid coding sequence with the bacterial *LacZ* and *neo* genes (Fig. 1a). Embryonic stem (ES) cells carrying the mutation were aggregated with morulas to produce chimaeras. Founder chimaeric males were mated to wild-type females, and their offspring showed the expected 1:1 ratio between +/- and +/+ animals. When +/- mice were interbred, no -/- offspring were found at birth (Fig. 1c; Table 1) or at any embryonic stage older than 10.5 days post-coitum (d.p.c.). At 10.5 d.p.c., all the -/- embryos and about half the +/- embryos were found dead (14 -/- and 18 +/- dead embryos; 25 +/- and 10 +/+ live embryos), whereas all the -/- and +/- embryos examined at 8.5 and 9.5 d.p.c. looked healthy (13 -/-, 19 +/- and 10 +/+ embryos were all alive). Thus, the *Mash-2*^{-/-} mutation is lethal at ~9.5–10.5 d.p.c. In addition, the observation of a similar phenotype in the homozygotes and half of the heterozygous progeny suggested that one of the parental *Mash-2* alleles may not be expressed in the embryo.

The phenotype of the *Mash-2*^{-/-} mutation was examined in the progeny of heterozygous intercrosses (F₂) between 7.5 and 10.5

TABLE 1 Genotypes of normal F₂ progeny and tetraploid aggregation chimaeras from *Mash-2*^{-/-} heterozygous intercrosses at birth

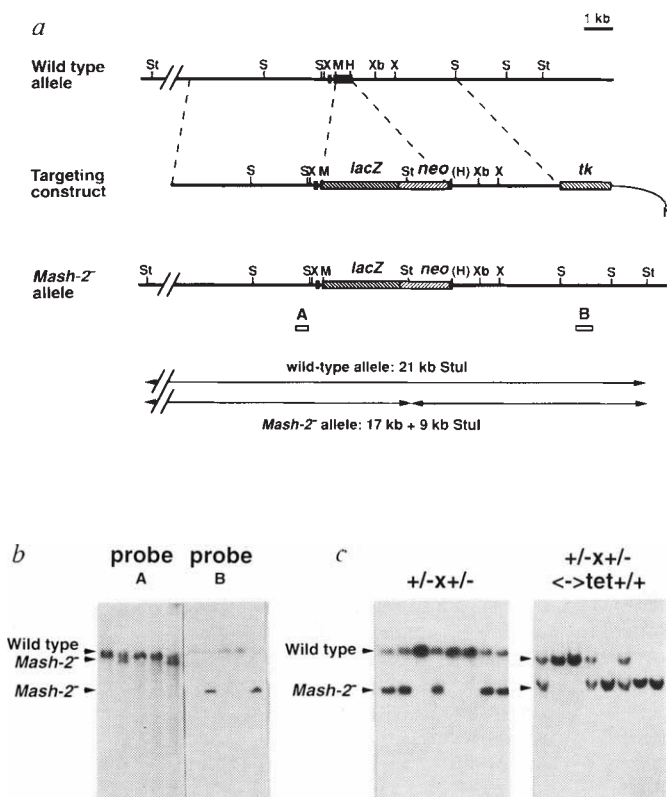
Crosses	-/-	-/+	+/+
-/+ × -/+	0 (0%)	62 (57%)	47 (43%)
-/+ × -/+ ↔ tetraploid	10 (15%)	28 (42%)	28 (42%)

d.p.c. Before they died at 9.5–10.5 d.p.c., embryonic tissues from homozygous mutant conceptuses were not distinguishable morphologically or histologically from their normal littermates (data not shown). In contrast, extraembryonic trophoblast cell types were clearly affected in mutant embryos from earlier stages. (Figs 2a–d and 3c, d). At 7.5 d.p.c., no obvious difference could be observed in the diploid cells of the chorion and the ectoplacental cone (EPC) between wild-type and mutant embryos. However, by 8.5 d.p.c., the EPC was devoid of diploid precursors. In 9.5- and 10.5-d.p.c. placentas, the spongiotrophoblast layer was completely missing and the labyrinthine layer did not have the highly vascularized organization of wild-type placentas. Chorion-allantoic fusion did occur, but the chorion retained its compact structure typical of earlier stages of development. In contrast with the elimination or reduction of the diploid trophoblast tissues, secondary giant cells were present and formed multiple cell layers instead of the discontinuous single-cell layer seen in normal placentas.

Expression of trophoblast differentiation markers was then examined in order to confirm these observations. Transcripts for genes 4311 (ref. 11) and Flt-1 (ref. 12) are restricted in the trophoblast to the spongiotrophoblast and its precursors in the EPC (Fig. 3g and data not shown). These two markers were completely absent in the trophoblast of *Mash-2* mutant embryos at 7.5 d.p.c., when they are normally first expressed, and at all later stages (Fig. 3h and data not shown). Thus, the absence of *Mash-2* affects a subset of trophoblast cells as early as 7.5 d.p.c. before there is any clear histological defect. In contrast, secondary giant cells expressing the specific markers placental lactogen-

FIG. 1 Generation and transmission of a mutation in *Mash-2*. a, Targeting strategy for deleting most of the *Mash-2* coding region. The two *Mash-2* exons are indicated as black boxes. The internal probe A and external probe B used for Southern blotting analysis are shown as open bars, and the diagnostic restriction fragments with their expected sizes as lines. H, *HpaI*; M, *MluI*; N, *NotI*; S, *SmaI*; St, *StuI*; X, *XhoI*; Xb, *XbaI*. b, Southern blot analysis of genomic DNA from double-resistant ES cell clones, digested with *StuI* and hybridized to probes A and B. The position of the restriction fragments characteristic for the wild-type and *Mash-2*^{-/-} alleles are shown on the left. Two of the 5 clones shown are correctly targeted. c, Southern blot analysis of the newborn progeny of heterozygous intercrosses. Left: no *Mash-2*^{-/-} mice (absence of the top band) were found among newborn F₂ animals. Right: *Mash-2*^{-/-} animals were present among the progeny of heterozygous intercrosses rescued by tetraploid aggregation. Tetraploid cell contribution to rescued *Mash-2*^{-/-} chimaeras was studied by hybridizing 10 µg *StuI*-digested genomic DNA to probe B. No wild-type signal was observed under conditions in which a signal could clearly be detected from 100 ng wild-type genomic DNA (data not shown).

METHODS. A targeting vector¹⁹ for *Mash-2* was constructed using 129 DNA by deleting a 510-bp *MluI*-*HpaI* DNA fragment (amino acids 20 to 189 of the *Mash-2* protein sequence⁴) and replacing it by the bacterial *LacZ* gene and SV40 poly (A) sequence cloned in frame with the *Mash-2* translated sequence and a PGK-*neo* cassette in the same transcriptional orientation, with 5.7 kb and 4.4 kb of homology 5' and 3' of the *lacZ*-*neo* sequence, respectively. The linearized construct was electroporated²⁰ into the ES cell line R1 (ref. 10). For b and c, genomic DNA from ES cell clones resistant to selection by G418 and gancyclovir (b) and from the tail of newborn F₂ animals (c) were digested with *StuI* and hybridized with the 3' internal probe A (b) and the 5' external probe B (b, c). Out of 122 double-resistant ES cell clones, 19 homologous recombinants were identified. Two targeted clones were aggregated with CD1 morulae¹⁷ to generate germ-line chimaeras.



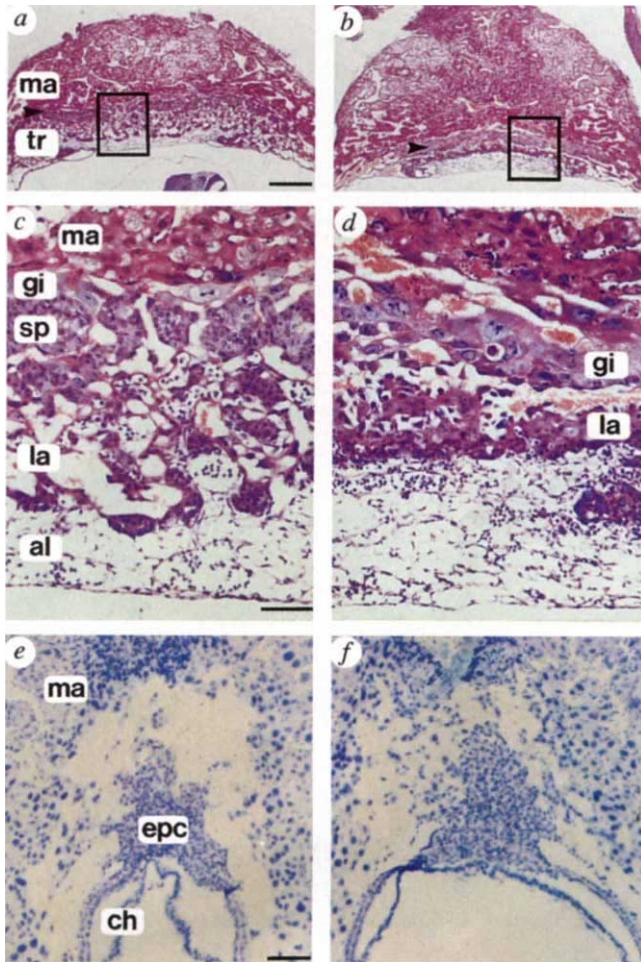


FIG. 2 Trophoblast tissues from normal (left panels) and *Mash-2* mutant (right panels) littermates. *a-d*, 9.5 d.p.c. placentas; *c* and *d* are higher magnifications of *a* and *b*, respectively. The spongiotrophoblast (sp) is absent and the labyrinthine trophoblast (la) is more compact in mutant than in normal placenta. Allantoic fusion has occurred, however, and some fetal blood vessels are apparent in the reduced labyrinthine area. The giant cell layer (gi) is enlarged. The same phenotype was observed in mutant animals derived from two independent targeted ES cell lines, and when the mutation was in an inbred 129Sv or outbred CD1 genetic background. *e, f*, Trophoblast tissues from 7.5-d.p.c. embryos. Trophoblast progenitors in the chorion (ch) and ectoplacental cone (epc) were present in both mutant and wild-type tissues. al, allantois; ma, maternal decidua. Scale bars, 500 μ m (in *a, b*); 100 μ m (in *c-f*).

METHODS. Embryos from heterozygous interbreedings were collected at 7.5–9.5 d.p.c. and processed for histology and *in situ* hybridization⁴. One section was used for RNA *in situ* hybridization⁴ with a *Mash-2* RNA antisense probe corresponding to the last 440 nucleotides of the translated sequence of the transcript and 80 nucleotides of 3' untranslated sequence, and *Mash-2*^{-/-} mutant embryos were identified by the absence of a hybridization signal. Other sections were processed for *in situ* hybridization with cell-type-specific probes (Fig. 3*i-l*) or stained with haematoxylin and eosin (2*a-d*) or toluidine blue (*e, f* and Fig. 3). There was complete correlation between lack of *Mash-2* hybridization signal and the mutant phenotype observed at 8.5 and 9.5 d.p.c.

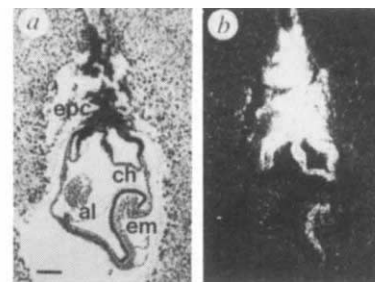


FIG. 3 Expression of *Mash-2* and cell-type-specific markers in trophoblast tissues. Normal (*a-c, e, g, i*) and mutant (*d, f, h, j*) embryos are at 7.5 (*a, b*) and 8.5 d.p.c. (*c-j*); *e, g, i* and *f, h, j* are near-adjacent sections. *c, d*, Bright-field images of the sections shown in *e* and *f*, respectively. *b, e*, RNA *in situ* hybridization with a *Mash-2* probe. *a-c, e*, *Mash-2* transcripts are present at a high level in the chorion (ch) and the EPC (epc), at lower level in the embryo (em) and are not detectable in secondary giant cells (gi) or the allantois (al). *d, f*, *Mash-2* transcripts are absent in mutant trophoblast. *g, h*, RNA *in situ* hybridization with a probe for the gene 4311 which marks spongiotrophoblast cells in the placenta and their precursors in the EPC¹¹; 4311-expressing cells are absent in mutant trophoblast. *i, j*, RNA *in situ* hybridization with a probe for the gene placental lactogen-1 which specifically marks trophoblast giant cells¹³. PL-1-expressing cells are present in mutant trophoblast. ma, Maternal decidua. Scale bar, 100 μ m. For methods, see Fig. 2 legend.

1 (PL-1; ref. 13) (Fig. 3i) and proliferin (ref. 14; data not shown) were found at all stages (Fig. 3j). Finally, expression of markers for the chorion and labyrinthine trophoblast, such as *tec* (ref. 15, and M. Brunkow, B. Motro and A. Bernstein, personal communication) and VEGF (ref. 16, G. Feng, D. Dumont and M. Breitman, personal communication) was still seen in mutant embryos between 8.5 and 10.5 d.p.c. (data not shown), although these tissues were reduced in later embryos.

The fact that *Mash-2*^{-/-} embryos proceed apparently normally through implantation shows that embryonic *Mash-2* expression is not required for generation of the trophectoderm at the blastocyst stage, nor indeed for the generation of the diploid trophoblast progenitors of the chorion and the EPC. The mutation appears to prevent primarily the transformation of these progenitors into 4311⁺ spongiotrophoblast precursors, which normally takes place mainly between 7.5 and 8.5 d.p.c. Additional *in vivo* and *in vitro* experiments will be necessary to determine in which of the processes of proliferation, survival or differentiation of trophoblast progenitors *Mash-2* function is primarily required. The presence of giant cells in mutants demonstrates that *Mash-2* is not required for the differentiation of this particular cell type.

Trophoblast was the only tissue that showed obvious developmental defects in *Mash-2*^{-/-} mutants, consistent with the high expression of the gene in this tissue. Death of the embryos at about 10 d.p.c. is also consistent with a primary placental failure, as this is the time when the chorio-allantoic circulation is established. But a later role for *Mash-2* in development of the embryo proper could not be excluded, as low-level expression was observed transiently in the embryo (Fig. 3b). To address this possibility, we rescued the placental phenotype of the *Mash-2*^{-/-} mutation by aggregating *Mash-2*^{-/-} diploid and wild-type tetraploid embryos^{9,10}. Tetraploid cells, which can contribute efficiently to all the extraembryonic tissues but not to the embryo itself, should complement the lack of normal trophoblast tissues in *Mash-2*^{-/-} embryos, allowing *Mash-2* function to be studied in the embryo. Morulas from *Mash-2*^{+/-} intercrosses were aggregated with 4-cell-stage wild-type CD1 tetraploid embryos. Chimaeras were transferred into foster mothers and genotyped at birth (Fig. 1b). Of the resulting viable offspring, 15% were *Mash-2*^{-/-} (Table 1), showing that wild-type tetraploid cells could successfully rescue the lethal placental phenotype of the *Mash-2*^{-/-} mutation. Furthermore, analysis of tail genomic DNA from *Mash-2*^{-/-} ↔ tetraploid chimaeras by Southern blot did not detect any signal corresponding to the *Mash-2*⁺ allele, showing that tetraploid cell contribution in *Mash-2*^{-/-} animals was less than 1% (see legend to Fig. 1). These results indicate that *Mash-2*^{-/-} cells can fully support embryonic development and *Mash-2* has no obvious embryonic function.

In conclusion, *Mash-2* is required at post-implantation stages for the generation of some of the differentiated trophoblast cell types. This is reminiscent of the role played by the other mouse *achaete-scute* homologue, *Mash-1*, in neuronal lineages¹⁸. The death of *Mash-2*^{-/-} embryos is probably due to failure to establish a proper chorio-allantoic circulation. It will be interesting to study how the human counterpart of *Mash-2* is expressed during normal pregnancy and whether this expression is affected in pathological conditions.

Embryonic transcription of *Mash-2* is not required for preimplantation development, but it is possible that maternal *Mash-2* transcripts or protein accumulate during oogenesis and play a role in the early embryo, for example in formation of the trophectoderm. The generation of adult *Mash-2*^{-/-} females by tetraploid rescue will allow us to directly test this hypothesis. □

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Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischaemia

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THE immunosuppressive action of the drug FK506 involves inhibition of calcineurin in T-lymphocytes by a complex of FK506 and an FK506 binding protein, FKBP12^{1,2}, a member of the immunophilin protein family^{3,4}. The functional role of brain immunophilins is, however, unclear^{5,6}. We show here that FK506 is a powerful neuroprotective agent in an *in vivo* model of focal cerebral ischaemia when administered up to 60 min post-occlusion. The minimum effective neuroprotective dose is comparable with the immunosuppressant dose in humans⁷, suggesting that FK506 may have clinical potential for the treatment of stroke. Although the related immunosuppressants rapamycin and cyclosporin failed to reduce brain damage, the finding that rapamycin pretreatment blocked the effect of FK506 confirms a role for immunophilins in the neuroprotective mechanism.

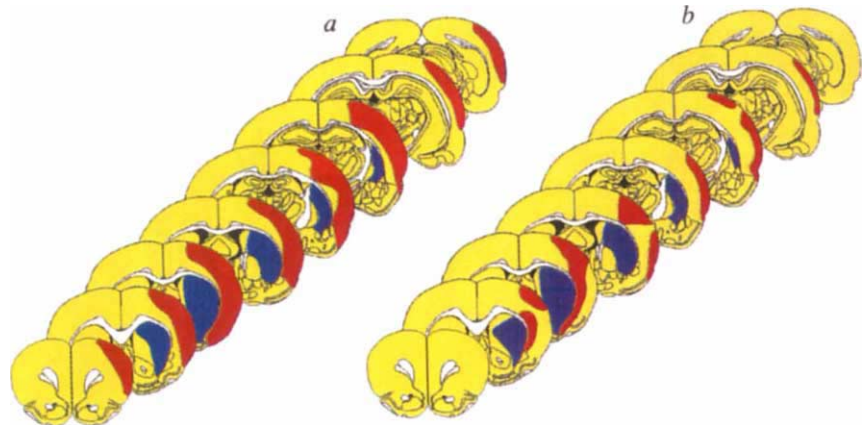
Animal models of focal cerebral ischaemia based on middle cerebral artery (MCA) occlusion reproduce the pattern of ischaemic brain damage observed in many human stroke patients. Damage extends throughout the vascular territory of the MCA^{8–10}: that is, within the striatum and cortex (Fig. 1). We studied the neuroprotective action of FK506 (tacrolimus, Fujisawa) in a rat MCA occlusion model of stroke, using intravenous (i.v.) administration of FK506 1 min post-occlusion. By this route, FK506 proved to be a powerful neuroprotective agent at doses of 0.1–1 mg per kg body weight (Fig. 2). The neuroprotective effect of the minimum effective dose of 0.1 mg kg⁻¹ was apparent in the cortex at each of the eight stereotactic levels examined (Fig. 1). Cortical damage was reduced by 62% (0.1 mg kg⁻¹), 58% (0.3 mg kg⁻¹) and 65% (1.0 mg kg⁻¹) compared with vehicle controls (Fig. 2). FK506 was, however, ineffective at a dose of 0.03 mg kg⁻¹, and striatal damage was

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FIG. 1 The pattern of cortical (red) and striatal (blue) damage observed at eight stereotactic levels in rat brains treated with vehicle (a) and 0.1 mg per kg body weight FK506 i.v. (b) 3 days following intracerebral (i.c.) microinjection of endothelin-1 adjacent to the middle cerebral artery.

METHODS. Male Sprague-Dawley rats (280–300 g) (Charles River) were anaesthetized with halothane in nitrous oxide:oxygen (1–2% in 80:20 v/v), and endothelin-1 (120 pmol in 3 μ l) injected over 3 min through a 29-gauge cannula stereotactically placed into the piriform cortex $\sim$ 0.5 mm dorsal to the middle cerebral artery (0.2 mm anterior and 5.2 mm lateral to bregma; and 7.5 mm below the dura). Rats received an i.v. injection of FK506 (0.03–1.0 mg kg⁻¹) or vehicle (10% ethanol containing 400 mg kg⁻¹ polyoxyl 60 hydrogenated castor oil in saline; 1 ml kg⁻¹) 1 min after completion of the endothelin injection. After a further 2 min the cannula was slowly withdrawn. Rats were maintained at 37–38 °C throughout the procedure using a thermostatically controlled heating blanket attached to a rectal thermometer. After surgery, animals were placed in an incubator in which body temperature was maintained at 37 °C until they had fully recovered from the anaesthetic. Three days after injection, the rats were re-anaesthetized with pentobarbitone and fixed by transcardial perfusion with paraformaldehyde (4% in PBS). The brain was removed intact and immersed in fixative containing 10% sucrose for at least 24 h before



cryostat sectioning at 20 μ m and histological staining with cresyl violet. Sections were examined by light microscopy by an individual who was blind to the treatment groupings. The extent of the infarction at eight predetermined levels was annotated onto diagrams of the brain, and the volume of infarction calculated by integrating the cross-sectional area of damage at each stereotactic level and the distances between the various levels⁹.

unaltered by any dose of FK506 tested (Fig. 2). The degree of neuroprotection was similar to that reported previously using the non-competitive NMDA receptor antagonist, MK801^{9,10}. The finding that brain damage is reduced in cortex but not striatum has been attributed to differences in the vascular supply to these brain areas¹¹, and is also typical of the actions of neuroprotective drugs tested in MCA occlusion models^{9,10}. Independent researchers (K. Katsuta and T. Takamatsu, personal communication) have confirmed the neuroprotective action of FK506 using the Tamura surgical model of rat MCA occlusion⁹. The total volume of brain infarction, assessed using the triphenyl-tetrazolium chloride (TTC) staining method, was reduced 30% by i.v. FK506 (0.32 mg kg⁻¹) and 32% by MK801 (0.32 mg kg⁻¹).

We also evaluated the pharmacological specificity of the neuroprotective action of FK506. The related immunosuppressants rapamycin (1 mg kg⁻¹, i.v.) and cyclosporin (1 mg kg⁻¹, i.v.) did not reduce the volume of ischaemic brain damage when given 1 min after vessel occlusion (Fig. 3). The neuroprotective action of FK506 was confirmed in this experiment, with 1 mg kg⁻¹ FK506 reducing cortical damage by 57%. As rapamycin and FK506 bind to the same immunophilin (FKBP12; relative molecular mass (M_r 12,000)) with similar affinities^{12,13}, the role of immunophilin binding in the neuroprotective action of FK506 was examined by pretreating animals with rapamycin before vessel occlusion and FK506 administration. Rapamycin administered i.v. 5 min before MCA occlusion blocked the neuroprotective action of FK506 given 1 min after MCA occlusion. Cortical damage was reduced by 45% ($P < 0.05$) in animals treated with FK506 (0.1 mg kg⁻¹), whereas in animals pretreated

with 1 mg kg⁻¹ rapamycin before administration of FK506 (0.1 mg kg⁻¹), a nonsignificant 7% reduction was noted. FKBP12 is therefore involved in the cellular mechanism underlying the neuroprotective action of FK506.

The temporal window of neuroprotective efficacy has also been examined. Cortical damage was reduced by 46% when

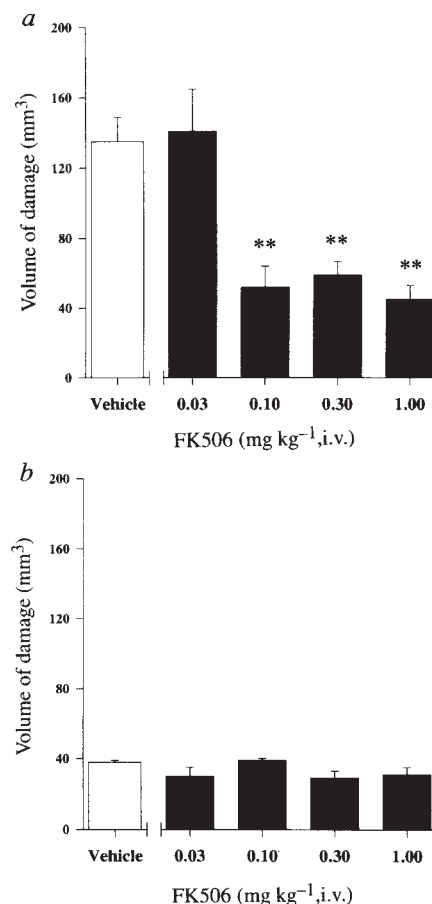


FIG. 2 Effect of i.v. FK506 on the volume of ischaemic damage in cortex (a) and striatum (b) when administered 1 min after MCA artery occlusion induced by i.c. injection of endothelin-1. Data are presented as mean volume of ischaemic damage (mm³) $\pm$ s.e.m. for nine animals per treatment group. Statistical analysis was performed using Student's *t*-test with Bonferroni correction for multiple comparisons (** $P < 0.01$). Significant reductions in the volume of cortical damage were observed at all but the lowest (0.03 mg kg⁻¹) dose of FK506. No significant alterations in the volume of ischaemic damage in the striatum were noted.

FIG. 3 Comparison of the neuroprotective effects of cyclosporin (CsA, 1 mg kg^{-1}), rapamycin (1 mg kg^{-1}) and FK506 (1 mg kg^{-1}) on the volume of ischaemic brain damage in cortex (solid bars) and striatum (open bars) produced by MCA occlusion. *a*, FK506 (1 mg kg^{-1}) reduces the volume of ischaemic brain damage in cortex but not striatum, whereas neither rapamycin nor cyclosporin alter the volume of brain damage. *b*, Pretreatment with rapamycin blocks the neuroprotective effect of FK506. Rapamycin (1 mg kg^{-1} , i.v.) was administered 5 min before injection of endothelin-1 with FK506 (0.1 mg kg^{-1} , i.v.) administered 1 min later.

METHODS. Immunosuppressants were administered i.v. 1 min after MCA occlusion. Rapamycin and cyclosporin were dissolved in the same vehicle used for FK506 (see Fig. 1 legend). Data are presented as mean volume of ischaemic damage (mm^3) $\pm$ s.e.m. for 7–13 animals per treatment group. Statistical ana-

lysis was performed using ANOVA, and where appropriate Tukey post-hoc comparisons were made.

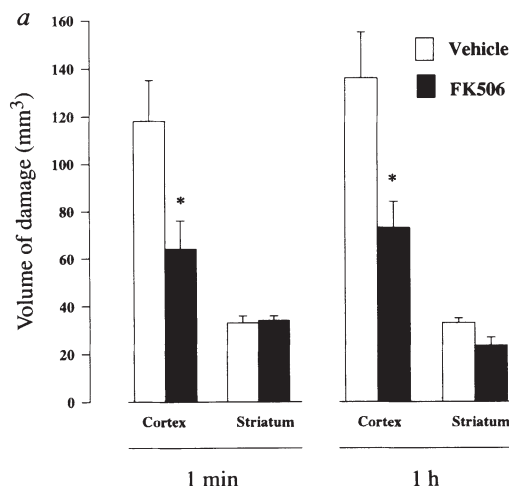
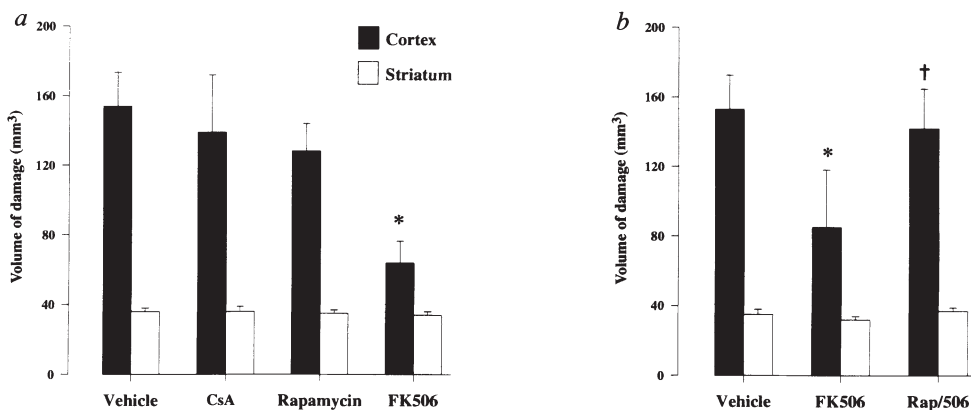
* $P < 0.05$ for the comparison between treatment and vehicle groups.

† $P < 0.05$ for the comparison between treatment groups.

FK506 (1 mg kg^{-1}) was administered 60 min post-occlusion ($P < 0.05$); striatal damage was unaffected (Fig. 4). In a parallel group of animals the effects of FK506 (1 mg kg^{-1}), MCA occlusion and a combination of these treatments on mean arterial blood pressure and partial oxygen tension ($p\text{O}_2$), partial carbon dioxide tension ($p\text{CO}_2$) and blood pH were determined. Neither drug nor MCA occlusion affected these parameters 60 min after treatment (Fig. 4); similar results were obtained at an intermediate 10 min time point (data not shown).

These data demonstrate that FK506 is a powerful neuroprotective agent in an *in vivo* model of focal cerebral ischaemia of equivalent efficacy to the non-competitive NMDA receptor antagonist, MK801^{9,10}. Neuroprotection was apparent when FK506 was administered 60 min post-occlusion, suggesting there is a temporal window for therapeutic intervention. As cardiovascular and respiratory parameters were unaffected by FK506 a direct neuronal effect is likely, and FK506 is reported to be neuroprotective in an *in vitro* neuronal culture model of excitotoxicity¹⁴. Binding of FK506 and the related immunosuppressants rapamycin or cyclosporin, inhibits the *cis-trans* peptidyl-prolyl isomerase (PPI) activity of relevant immunophilins^{15–17}. But as neither rapamycin nor cyclosporin reduces ischaemic brain damage, inhibition of PPI is unlikely to be of direct importance. Rapamycin pretreatment blocked the effect of FK506, however, suggesting that immunophilin binding is an essential step in the neuroprotective mechanism. FK506 and rapamycin compete for a common binding site on FKBP12^{12,13}, suggesting that inhibition of the protein phosphatase, calcineurin is implicated in the neuroprotective effect. Calcineurin involvement would explain the lack of rapamycin efficacy because a complex of FK506-FKBP12, but not rapamycin-FKBP12, inhibits calcineurin^{18,19}. Calcineurin is also present in the brain^{20,21}. The finding that cyclosporin was ineffective is therefore surprising because the cyclosporin-cyclophilin complex also inhibits calcineurin^{18,19}. As subchronic pretreatment with high doses of cyclosporin is reported to reduce brain oedema following focal cerebral ischaemia²², the lack of efficacy in this study may reflect the low blood-brain barrier permeability of cyclosporin following a single injection²³.

Several intracellular processes could account for the proposed role of calcineurin in neurodegeneration. This enzyme is reported to regulate calcium channel activity²⁴, and a central role for calcium in neurodegeneration has been proposed²⁵. Alternatively, a mechanism involving the inhibition of free radical production may be involved, as has been postulated for the protective properties of FK506 in animal models of myocardial



b

	Vehicle		FK506	
	Baseline	1 h post	Baseline	1 h post
Temperature ($^{\circ}\text{C}$)	37.2 ± 0.2	36.9 ± 0.1	37.0 ± 0.2	36.8 ± 0.1
MABP (mmHg)	84 ± 4	80 ± 1	84 ± 4	82 ± 1
$p\text{CO}_2$ (mmHg)	46 ± 2	39 ± 3	47 ± 3	46 ± 3
$p\text{O}_2$ (mmHg)	104 ± 9	109 ± 10	112 ± 8	87 ± 12
pH	7.42 ± 0.01	7.44 ± 0.02	7.43 ± 0.01	7.44 ± 0.04

FIG. 4 *a*, Effects of FK506 (1 mg kg^{-1} , i.v.) on the volume of ischaemic brain damage when administered either 1 min or 60 min following i.c. injection of endothelin-1 adjacent to the rat MCA. A significant reduction ($P < 0.05$) in the volume of cortical brain damage is observed in FK506-treated rats at both time-points using this injection protocol. *b*, Effect of FK506 upon physiological parameters measured immediately before (baseline), and at 1 h after, the i.c. injection of endothelin-1 adjacent to the rat MCA. Values for mean arterial blood pressure (MABP), partial oxygen tension ($p\text{O}_2$), carbon dioxide tension ($p\text{CO}_2$) and pH in arterial blood are presented as mean $\pm$ s.e.m. Statistical analysis (two-way ANOVA) revealed no significant differences between FK506 and vehicle treatment at either time point or between the pre- and post-endothelin values within any treatment group. Similar findings were obtained at intermediate timepoints (for example 10 min, data not shown).

ischaemia²⁶. Indeed, FK506 has been shown to inhibit superoxide production in neutrophils²⁶. FK506 has recently been shown to reduce nitric oxide (NO) production by inhibiting the calcineurin mediated dephosphorylation of nitric oxide synthase¹⁴. This finding provides a link between the FK506-FKBP12 complex, calcineurin and free radical production involving NO. Nitric oxide is proposed to mediate the neurotoxic properties of excitatory amino acids and NO donors^{27,28}, and the neurotoxic properties of free radicals produced by the interaction of NO and superoxide anions has recently been reported²⁹. □

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Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast

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A YEAST cell becomes committed to the cell division cycle only if it grows to a critical size^{1–4} and reaches a critical rate of protein synthesis^{5,6}. The coordination between growth and division takes place at a control step during the G1 phase of the cell cycle called Start⁴. It relies on the G1-specific cyclins encoded by *CLN1*, 2 and 3, which trigger Start through the activation of the Cdc28 protein kinase. In fact, the Cln cyclins are rate-limiting for Start execution and depend on growth^{7–10}. Here we report that the cyclic AMP signal pathway¹¹ modulates the dependency of Cln cyclins on growth. In particular, more growth is required to trigger Start because *CLN1* and *CLN2* are repressed by the cAMP signal, thus explaining the previously observed cAMP-dependent increase of the critical size and critical rate of protein synthesis¹². Cln3 is not inhibited by the cAMP pathway and counteracts this mechanism

by partially mediating the growth-dependent expression of other G1 cyclins.

We have developed a sensitive test (the α /–N test) to monitor the cAMP effect on Start (Fig. 1a). Cells were arrested at Start with α -factor⁴, then after removing α -factor, the rate of protein synthesis was limited by nitrogen starvation (–N) so that the resulting rate was below the critical rate of protein synthesis induced by a cAMP pathway hyperactivation, but was still

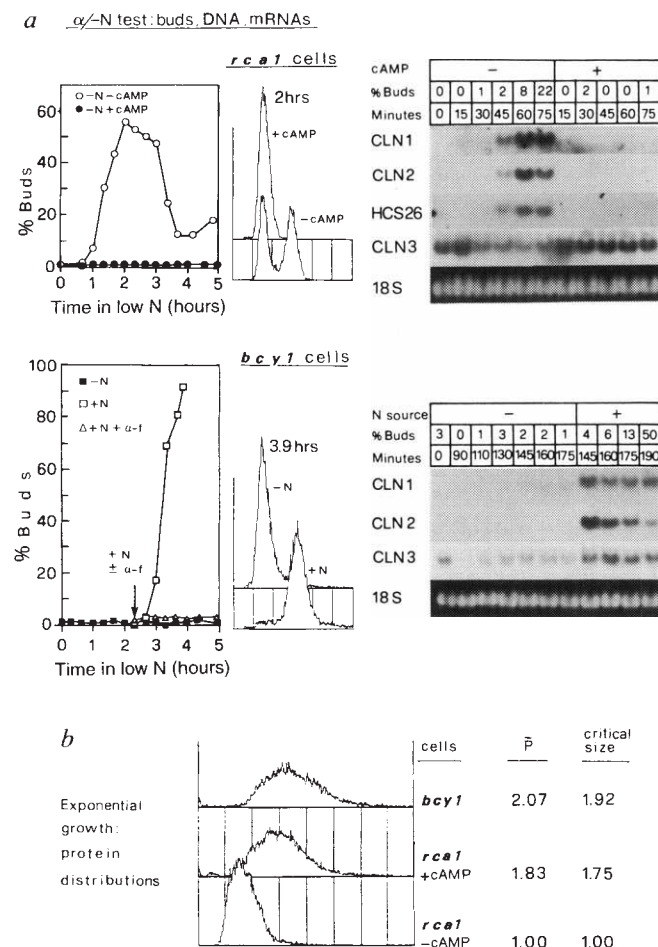


FIG. 1 Start inhibition by cAMP pathway involves repression of *CLN1*, *CLN2* and *HCS26*, but not *CLN3*, expression and it is suppressed by growth. **a**, Per cent of budded cells, flow-cytometric DNA distributions and northern analyses of *CLN* mRNAs in *rca1* and *bcy1* cells undergoing the α /–N test (see text for rationale). Cells (strains RS-16B [*MATa ade1 his3 leu2 rca1 trp1 ura3*] and RSBC1 [*MATa ade1 bcy1::URA3 his3 leu2 rca1 trp1 ura3*]) growing in YNB/glucose were pre-synchronized at Start by incubation for 2 h with α -factor. At time zero, α -factor was washed out and cells were resuspended into a low-nitrogen glucose medium (–N) (YNB without ammonium sulphate, 50 mg ml^{–1} adenine, 10 mg ml^{–1} uracil (only for *Ura*[–] cells), 0.5 mg ml^{–1} histidine, leucine and tryptophan) with (●) or without (○, ■) the addition of a 3 mM concentration of cAMP. After 140 min (arrow), 5 mg ml^{–1} of (NH₄)₂SO₄ and 50 mg ml^{–1} of the auxotrophic requirements were added to *bcy1* cells without (□) or with (△) 2 μ M α -factor (+N $\pm$ α -f.). Samples for flow cytometry or RNA extraction were taken at the indicated times. **b**, Flow cytometric protein distributions and critical size of strains during exponential growth on YNB/glucose. $\bar{P}$ is the normalized mean of the distributions.

METHODS. RSBC1 derives from RS-16B after transformation with p73a plasmid¹⁴ cut with *Bam*H1. RNA extraction and FACS analysis were performed as in refs 17 and 12, respectively. *CLN* mRNAs were detected with Digoxigenin (DIG) labelled riboprobes (DIG Luminescent Detection Kit for Nucleic Acid; Boehringer). Each lane was loaded with 6 μ g total RNA. The critical size was calculated from $\bar{P}$ as described¹².

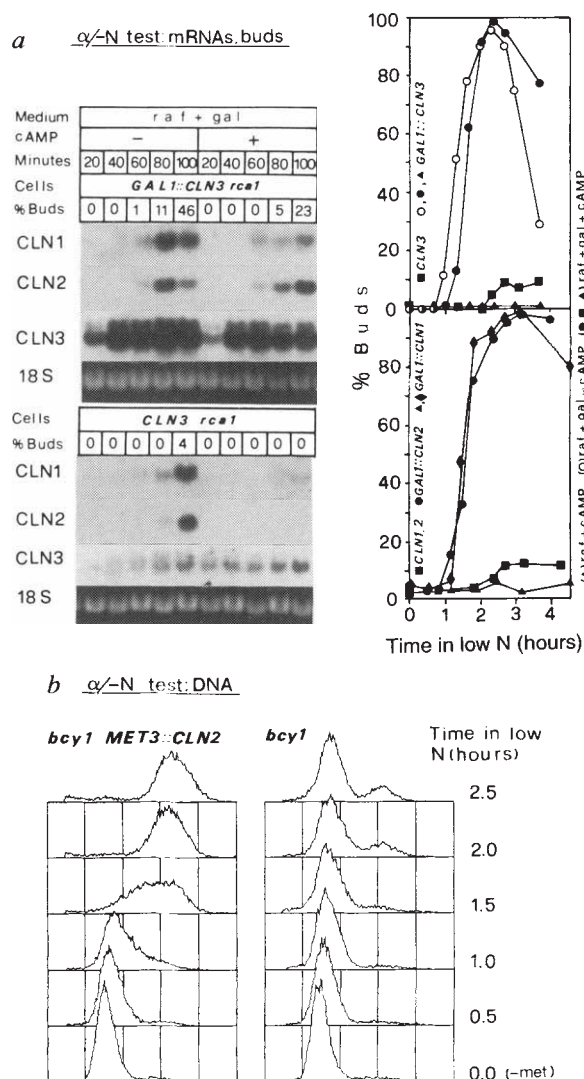


FIG. 2 Overexpression of *CLN1*, *CLN2* or *CLN3* suppresses Start inhibition induced by cAMP pathway. **a**, Northern analysis and per cent of budded cells of *GAL1::CLN3 rca1* cells. Performing the α -N test, the *rca1* cells were treated with α -factor in YNB/raffinose whereas the final low-nitrogen medium contained raffinose (2%) or, alternatively, raffinose plus galactose (2%). 3 mM cAMP was added (filled symbols) at time zero. Strains, isogenic to RS-16B, were: RSG13 (*cln3::GAL1::CLN3 URA3 rca1*) (○, ●, ▲) and RSC3U (*CLN3 URA3 rca1*) (■) (upper panel and northern analysis); RSG12 (*LEU2::GAL1::CLN2 rca1*) (●), RSG11 (*LEU2::GAL1::CLN1 rca1*) (▲, ◆) and RSCL (*CLN1 CLN2 LEU2 rca1*) (■) (lower panel). DNA distributions (not shown) confirmed cell cycle commitment of *GAL1::CLN* cells in galactose. **b**, Flow-cytometric DNA distributions of RSBC1-M2 strain cells (*bcy1::URA3 TRP1::MET3::CLN2*) and isogenic RSBC1-T cells (*bcy1::URA3 TRP1*) subjected to the α -N test in YNB/glucose. 65 μ M exogenous methionine was added during exponential growth and α -factor treatment, but was omitted in the low-nitrogen medium. Samples for DNA analysis were taken at the indicated times. The budding index confirmed that the *bcy1* MET3::CLN2 cells were committed to the cell cycle (not shown). The small fraction of control *bcy1* cells accumulated in G2 + M phase did not further increase (not shown).

METHODS. RS-16B cells were transformed with the following plasmids: pFP5-2 (provided by B. Futcher) (cut with *PvuII* + *EcoRI*) to obtain the *GAL1::CLN3* cells; pM212 (cut with *KpnI*) and pIGC1 (cut with *HpaI*) (provided by F. Cross) to obtain the *GAL1::CLN2* and the *GAL1::CLN1* cells, respectively. Strain RSBC1 (Fig. 1 legend) was transformed with p2436 (gift from K. Nasmyth) (cut with *BstXI*) to construct the MET3::CLN2 cells.

sufficient to trigger Start in normal cells¹². Using this approach, either the cAMP addition to *rca1* cells (bearing a defective cAMP phosphodiesterase¹³) or the inactivation of *BCY1* (encoding the regulatory subunit of A-kinases¹⁴) permanently prevented the execution of Start. The block at Start must be due to an increased growth requirement as it could be fully reversed just by adding a non-limiting concentration of nitrogen source (+N); in addition, during the exponential growth both treatments caused a large increase in the critical size (Fig. 1b).

Northern analysis showed that *CLN1* and *CLN2* messenger RNAs were virtually undetectable in both *rca1* cells treated with cAMP and in starved *bcy1* cells (Fig. 1a). Repression of *CLN1*

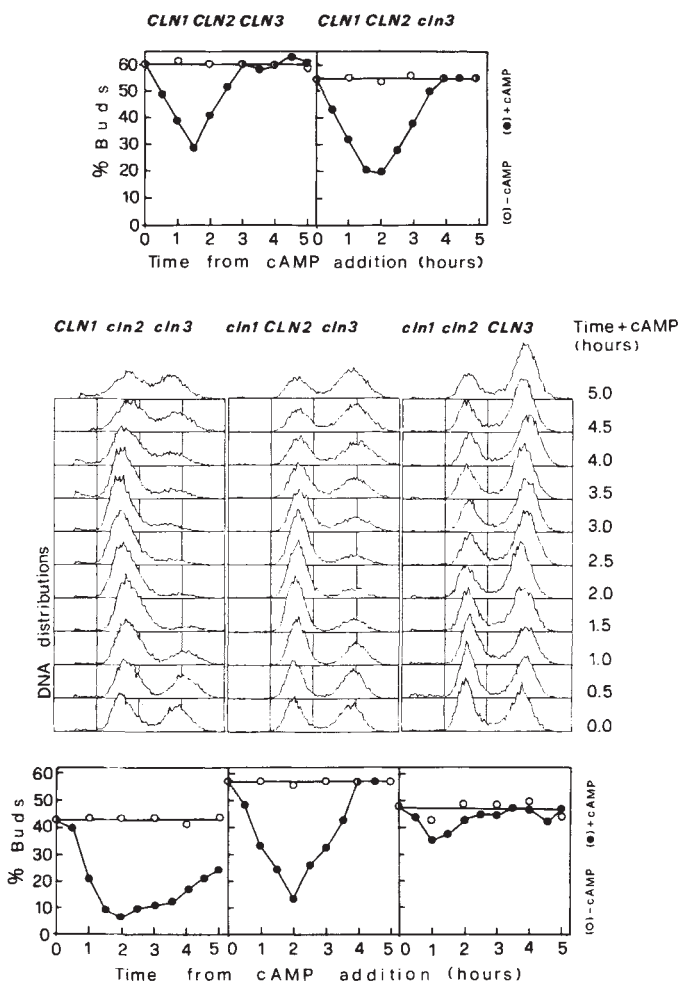


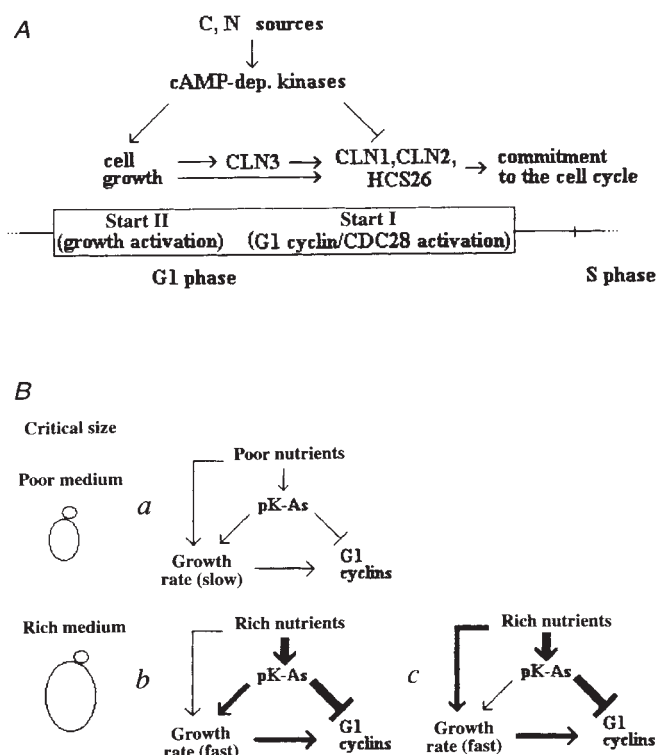
FIG. 3 In exponentially growing cells cAMP produced a prolonged pause at Start when *CLN3* is missing. Per cent of budded cells and DNA distributions of populations exponentially growing on YNB/glucose (○) and after addition of 3 mM exogenous cAMP (●; DNA). cAMP was added (time zero) at a cell density of $1-2 \times 10^6$ cells per ml. Strains and relevant genotypes were: RS-16B (*CLN1 CLN2 CLN3*), RSD-3U (*CLN1 CLN2 cln3::URA3*), RSD-2L3U (*CLN1 cln2::LEU2 cln3::URA3*), RSD-1D3U (*cln1::HisG CLN2 cln3::URA3*), RSD-1U2L (*cln1::HisG::URA3 cln2::LEU2 CLN3*). Samples for flow-cytometric analysis were taken at the indicated times.

METHODS. Double gene disruptions were directly obtained by two sequential transformations of RS-16B cells with the following plasmids: pWJ310 (ref. 8; cut with *HpaI* + *SphI*) for the *cln3::URA3* disruption, pJH3-45-2 (ref. 10; cut with *Sall* + *HindIII*) for the *cln2::LEU2* disruption and pC1599 (provided by K. Nasmyth) (cut with *EcoRI*) for the *cln1::HisG* disruptions. At least three clones of each strain were analysed and showed an identical response to cAMP.

FIG. 4 cAMP pathway helps to regulate critical cell size required at Start by repressing G1 cyclin expression. **A**, Yeast cells become committed to the cell division cycle at a regulatory step during G1, called Start⁴. A block at Start II (a quiescent-like state) is the result of a growth restriction. Activation of cAMP-dependent protein kinases (pK-As) is necessary for both growth and Start II execution^{11,21-23,26,28} and depends on nutrient availability. Carbon sources (C) (in particular glucose) can stimulate adenylate cyclase activity while nitrogen sources (N) might more directly activate pK-As without varying intracellular cAMP levels (discussed in ref. 28). Growth and division are coordinated at Start I, that can be triggered only if a yeast cell can reach a critical rate of protein synthesis (CRPS)⁶. The availability of yeast G1 cyclins (CLNs) is limiting for Start I execution (via CDC28 kinase activation) and depends on continuous growth^{7-10,16,17,19,20,29}. This explains the CRPS requirement at Start I. An increase of pK-A activity raises the CRPS required to trigger Start I by repressing *CLN1*, *CLN2* and at least another putative G1 cyclin gene, *HCS26* (ref. 15). *CLN3* counteracts this mechanism by partially mediating the growth-dependent expression of the other G1 cyclins. This model does not exclude that cAMP can also stimulate the CLNs (especially *CLN3*) by a growth-independent mechanism as suggested by the increase of *CLN3* expression in the $\alpha/-N$ test (Fig. 1a) and by a recent report³⁰. Other roles of *CLN3* in cell-cycle commitment (discussed in ref. 29) are not included within this scheme. **B**, The cell size at which Start is triggered (critical size) is remarkably constant under most growth conditions and increases at the fastest growth rates^{2,3}. As the rate of protein synthesis of a single cell is proportional to both its size and specific growth rate, to achieve this critical size control the CRPS must be continuously raised in accordance with the growth rate. The degree of G1 cyclins inhibition by pK-As appears to be crucial to maintenance of this size control, since it can be proportional to growth rate. This fact is evident if pK-A activity is limiting for growth rate (a, b). However, only a basal pK-A activity may be required for growth^{12,26,28,31}. Nevertheless, even when a higher pK-A activity is unable to affect growth rate this increase can be a response to nutritional changes that somehow lead to a faster growth (a, c). In both hypotheses, pK-As can counterbalance differences in G1 cyclin stimulation caused by growth rate changes and helps to regulate the critical size. In the models, a rise in the critical size can be simply explained by a prevalence of the pK-A-induced G1 cyclin repression with respect to the growth dependent stimulation. Models A and B are supported

and *CLN2* together with *HCS26* (another G1 cyclin-encoding gene¹⁵; Fig. 1a) can explain the arrest at Start. Indeed, *cln1cln2hcs26* cells can be very sick or even inviable (discussed in ref. 16). In addition, the overexpression of either *CLN1* or *CLN2* from the galactose-inducible *GAL1* promoter¹⁷ complemented the cAMP-induced inhibition of Start. This was observed with the $\alpha/-N$ test, pre-incubating cells with α -factor in raffinose (*GAL1* promoter off) and shifting them to a low-nitrogen medium containing raffinose or, alternatively, raffinose plus galactose (*GAL1* promoter on). In raffinose, the *GAL1::CLN* cells and the isogenic wild-type strain were arrested at Start by cAMP addition, whereas in galactose only the *GAL1::CLN1* and *GAL1::CLN2* cells were able to bud and enter the S phase (Fig. 2a, and data not shown). Similarly, the arrest at Start due to the *bcy1* mutation could be complemented by overexpressing *CLN2* from the methionine-repressible promoter *MET3* (ref. 18; Fig. 2b).

The inhibition exerted by the cAMP pathway was somewhat specific for *CLN1*, *CLN2* and *HCS26*, because the *CLN3* mRNA levels were slightly increased by cAMP addition. These results agree with a previous finding that in cAMP-deficient cells the levels of *CLN1*, *CLN2* and *HCS26* mRNAs are high, while *CLN3* expression is partially repressed¹⁶. Nevertheless, the overexpression of *CLN3* from the *GAL1* promoter could also suppress the block at Start of cAMP-treated cells. We excluded the possibility that Start was triggered before cAMP was sensed by the cells, because the supply of galactose either with cAMP or 80 min later had an identical effect (Fig. 2a, and data not shown). The suppression was preceded by the recovery of *CLN1*, *CLN2* and *HCS26* expression (Fig. 2a, and data not shown), suggesting that overexpression of *CLN3* does not bypass their requirement at Start, but that *CLN3* can interact more specifi-



by the finding that cells with a defective cAMP pathway (such as *cdc25* and *bcy1tpk1¹²tpk2tpk3* cells)^{11,26} are smaller than wild type in rich medium; in addition, *cln1cln2CLN3* cells can poorly modulate their cell size with nutritional conditions with respect to wild type, *CLN1cln2cln3* or *cln1CLN2cln3* cells (ref. 27, and unpublished results). $\rightarrow$, Stimulation; $\dashv$, inhibition. The thickness of the lines represents intensity of effects.

cally with the cAMP-mediated mechanism, apparently by mimicking the growth effect. This hypothesis is supported by the observation that turning on *GAL1::CLN3* in *cln1cln2GAL1::CLN3* cells can very poorly overcome the block to budding caused by cAMP (data not shown). Previous evidence also indicates that *CLN3* is a growth-sensitive element⁷⁻⁹ that can promote Start at least partially by stimulating the expression of other G1 cyclins^{17,19,20}.

The interaction between the cAMP pathway and the *CLN* genes was further analysed by adding cAMP to exponentially growing cells bearing various combinations of *cln* mutations. As expected, in continuously growing cells the cAMP-mediated inhibition of Start and *CLN* expression was transient (ref. 12, and data not shown). In these experiments, *CLN1CLN2cln3* cells, but not *cln1CLN2CLN3* or *CLN1cln2CLN3* cells, showed a more prolonged pause at Start than did wild-type cells. An even more dramatic pause at Start was seen when *CLN1cln2cln3* or *cln1CLN2cln3* cells were challenged with cAMP, whereas cAMP had little effect on *cln1cln2CLN3* cells (Fig. 3, and data not shown). Thus, cAMP produced a prolonged pause whenever *CLN3* was missing. This suggests, and is supported by northern analysis, that *CLN1* and *CLN2*, but not *CLN3*, are repressed by cAMP and that *CLN3* is required to mediate part of the growth effect as a suppressor of the cAMP-induced G1 arrest. In contrast, when *CLN1* and *CLN2* are missing, the cAMP effect can even be reduced, further supporting the idea that it partially relies on these elements. Presence of other cAMP-sensitive element(s), such as *HCS26*, can explain the residual inhibition of G1/S transition in fast-growing *cln1cln2CLN3* cells, although these element(s) appear less responsive to cAMP than *CLN1* and *CLN2*. The complete inability of *cln1cln2CLN3* cells to bud when subjected to the $\alpha/-N$ test in presence of cAMP (data

not shown) confirms that they still contain cAMP-sensitive element(s) that can trigger Start.

The distinction between Start II and Start I helps to reconcile our results with the previous finding that cAMP-deficient cells arrest at Start (Fig. 4A and legend). In fact, both starved and cAMP-deficient cells arrest at Start II with a growth lesion, whereas cells unable to trigger Start I show an impaired CLN/CDC28 system but grow well^{4,21-24}. The regulation of Start we describe here involves Start I and requires additional growth (in part mediated by CLN3) to be overcome. Thus, although the cAMP signal is necessary and possibly limiting for growth^{4,11,21-23,25}, it increases the growth required to push the cells into a new division cycle. Why is this device needed? The

rate of protein synthesis of a single cell is proportional to both its size and specific growth rate. So, the critical rate of protein synthesis appears to be continuously modulated with the growth rate as the yeast critical size is remarkably constant under most growth conditions and increases at the fastest growth rates. In contrast, a partial cAMP-pathway inactivation allows fast-growing cells to bud with a small critical size; moreover, the size of both *bcy1* and *bcy1tpk1^wtpk2tpk3* cells (bearing only one attenuated A-kinase catalytic subunit²⁶) is not properly modulated in different nutritional conditions (ref. 27, and unpublished results). We propose that the cAMP signal resets the critical rate of protein synthesis as a function of the nutritional environment and growth rate by inhibiting the growth-dependent expression of the G1 cyclins (Fig. 4B). □

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Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast

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In the yeast *Saccharomyces cerevisiae*, commitment to cell division (Start) requires growth to a critical cell size^{1–3}. The G1 cyclins Cln1, Cln2 and Cln3 activate the Cdc28 protein kinase and are rate-limiting activators of Start^{4–6}. When glucose is added to cells growing in a poor carbon source, the critical cell size required for Start is reset from a small to a large size^{2,3,7}. In yeast, glucose acts through Ras proteins to stimulate adenylyl cyclase, activating the three cyclic AMP-dependent protein kinases Tpk1, Tpk2 and Tpk3 (refs 8, 9). We find that stimulation of the Ras/cAMP pathway represses expression of *CLN1*, *CLN2* and co-regulated genes,

inhibiting Start. This helps explain the increase in critical size when cells are shifted from poor to rich medium. This connection between the molecules controlling growth (Ras/cAMP) and those controlling division (cyclins) helps explain how division is co-ordinated with growth.

Addition of glucose to cells growing in raffinose caused a severe but temporary drop in *CLN1* messenger RNA and protein levels (Fig. 1), and also in the histone H1 kinase activity associated with Cln1 (data not shown). Budding and analysis by fluorescence-activated cell sorting (FACS) showed the cells paused briefly in G1, and reset to a slightly larger cell size (from 36 to 41 μm^3). There was little effect on the expression of *CLN2* and *CLN3*.

The addition of glucose also caused a brief but pronounced increase in intracellular cAMP (Fig. 2a), similar to the increase seen when glucose is added to cells growing in even poorer carbon sources¹⁰. To investigate whether this spike of cAMP might be responsible for the loss of *CLN1* mRNA and protein, we examined a so-called 'wimp' strain, which lacks a functional Ras/cAMP signalling pathway but is viable because of a weak but constitutively active cAMP-dependent protein kinase encoded by *tpk1^w* (ref. 11). When glucose was added to wimp cells grown in raffinose, it failed to alter levels of *CLN1* mRNA (not shown) or protein (Fig. 2d). Therefore, a functional Ras/cAMP pathway is necessary for the effect of glucose on *CLN1*.

If this signalling pathway is responsible for nutrient modulation of critical size, then *TPK1 tpk2 tpk3 bcy1* cells (that is, cells with constitutively high levels of Tpk1 cAMP-dependent kinase; Bcy1 is the regulatory subunit of the kinases) should have low levels of Cln1 and large cell sizes in both good and poor media. Furthermore, *tpk1^w tpk2 tpk3 bcy1* cells (wimps) should have moderate levels of Cln1 and moderate cell sizes in both good and poor media. That is, both strains should lack the wild-type

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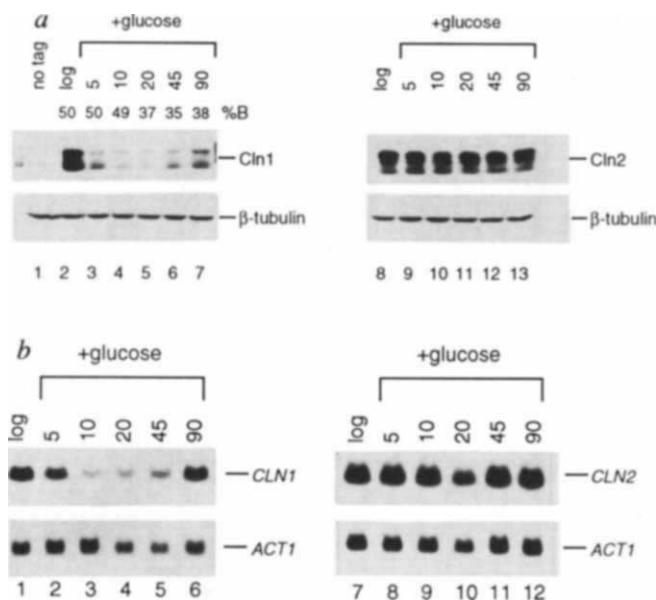


FIG. 1 Glucose represses *CLN1*. Glucose was added to cells growing in raffinose medium, and levels of *CLN1* and *CLN2* mRNA, protein and associated histone H1 kinase activity (not shown) were measured. **a**, Immunoblot showing Cln1 and Cln2 protein levels. Lane 1 contains lysate from an isogenic strain lacking haemagglutinin (HA) epitope tag; lanes 2 and 8 contain lysates from HA-tagged *CLN1* (*CLN1C*) and *CLN2* (*CLN2C*) cultures²⁹ respectively, before the addition of glucose. Lanes 3 to 7 (*CLN1C*) and 9 to 13 (*CLN2C*) are samples taken at the indicated times (min) after addition of glucose. '% B', per cent budding (average from three experiments, including the one shown). Below is the same blot probed for β -tubulin as a loading control. **b**, Northern blot showing *CLN1*, *CLN2* and *ACT1* mRNA levels. Lanes 1 and 7 contain total RNA from *CLN1C* and *CLN2C* cultures²⁹, respectively, before addition of glucose. Lanes 2 to 6 (*CLN1C*) and 8 to 12 (*CLN2C*) are samples taken at the indicated times (min) after addition of glucose.

METHODS. All strains were isogenic with W303 (*MATa ade2 leu2 his3 trp1 ura3 ssd1-d*). Cells carrying HA-tagged CLNs (*CLN1C*, GT104, or *CLN2C*, strain MT263; ref. 29) were grown in YEP + 2% raffinose to a density of 1×10^7 cells per ml, at which time a control sample was removed. The remaining culture was split in two, glucose or galactose was added to 2% and samples were taken after the indicated times. Galactose addition had no effect (data not shown). Immunoblot analysis, histone H1 kinase assays and northern blot analysis were performed as described²⁹. Probes for northern analysis were a 708-bp *NcoI*–*EcoRI* *CLN1* fragment, a 637-bp *SpeI*–*HindIII* *CLN2* fragment, and a 600-bp *EcoRI*–*HindIII* fragment of the *ACT1* gene.

ability to modulate cell size according to growth conditions. These predictions are correct (Fig. 2c, d, e).

If the cAMP pathway accomplishes these effects on size partly by regulating *CLN1*, then a *cln1*-deletion mutant should be partly defective in size regulation. We found that the critical size for budding increased by 9.5% for wild-type cells shifted from ethanol to glucose medium (from 28.5 to $31.2 \mu\text{m}^3$), but the increase was only 1.8% (34.1 to $34.7 \mu\text{m}^3$) for *cln1* cells (Fig. 2b). Similarly, in asynchronous cultures, the mode of the cell volume distribution increased by 15% when wild-type cells were shifted from ethanol to glucose medium (38 to $43.7 \mu\text{m}^3$), but the increase was only 3.3% (42.7 to $44.1 \mu\text{m}^3$) for *cln1* cells (not shown).

A *pde2* mutant strain responds to exogenous cAMP because it lacks the low- K_m phosphodiesterase^{12,13}. Addition of cAMP to *pde2* cells caused a rapid loss of Cln1 protein (Fig. 3b) and mRNA (Fig. 4) (note that the bands in lanes 6, 7 and 8 of the cAMP panel are not Cln1; see Fig. 3 legend). Therefore, cAMP is sufficient to cause the same effect as glucose. Most cells arrested or paused as unbudded cells in G1 but grew dramatically in size (Fig. 3a, and data not shown). Overproduction of *TPK1* from the *GAL1* promoter also caused loss of *CLN1* mRNA, protein, and associated kinase activity (data not shown).

We asked whether the cAMP-induced G1 pause could be suppressed by extra *CLN3* expressed from a galactose-inducible promoter. When a *pde2* strain containing *GAL1-CLN3* was grown in YEP plus 2% raffinose, 50% of the cells were budded. These cells were shifted to three other conditions for five hours, and budding and size were then assayed. In YEP plus 2% raffinose and 5 mM cAMP, 30% of cells were budded; in YEP plus 2% raffinose and 1% galactose, 70% were budded; and in YEP with 2% raffinose, 1% galactose and 5 mM cAMP, 75% were budded, showing that *GAL1-CLN3* suppresses the effect of cAMP on budding. The increase in cell size was also largely but not entirely suppressed. *GAL1-CLN1* had similar effects (not shown). However, cells in galactose and 5 mM cAMP were fragile and had abnormal morphologies, indicating that cAMP had additional effects not suppressed by extra *CLN3* or *CLN1*. These results are consistent with previous evidence that stimulation of the cAMP pathway by mutations or by addition of cAMP inhibits

Start and increases cell size, whereas partial down-regulation of the pathway decreases cell size^{14–16}.

CLN1 transcription is dependent on the transcription factor SBF, which contains Swi4 and Swi6 (refs 17–19). Many genes expressed in G1 and S are dependent on SBF or the related factor MBF^{20–23}. Figure 4 shows that many genes regulated by SBF or MBF are repressed by addition of cAMP to a *pde2* strain. In contrast, *CLN3* and *ACT1*, which are independent of SBF and MBF, were not downregulated. *CLN1* seemed to be more sensitive to cAMP than the other genes examined. Raffinose is a relatively good carbon source, so the shift from raffinose to glucose may only weakly stimulate the pathway. Perhaps that is why *CLN2* fails to respond to this nutritional shift, even though it responds to cAMP addition.

There are at least two possible mechanisms for this cAMP-mediated repression. cAMP could directly inhibit both SBF and MBF. Alternatively, cAMP could inhibit the Cln–Cdc28 kinase activities, which are required for activation of SBF and MBF^{24,25}. Because *CLN* mRNA, protein and kinase levels are interdependent^{24,25} it has been difficult to distinguish between these possibilities. Preliminary experiments suggest that *CLN1* mRNA and protein are each targeted by a cAMP-dependent mechanism.

G1 cyclin abundance is controlled largely by the rate of protein synthesis. Cyclic AMP is needed for growth and protein synthesis, and so indirectly stimulates Cln synthesis. However, it is now apparent that cAMP also has an opposite effect—it specifically antagonizes Cln expression by some mechanism. The temporary loss of Cln in response to cAMP can explain the Start delay when cells are shifted to rich medium. Thus, nutrient conditions may be coupled to cell-cycle progression by direct control of cyclin expression.

Steady-state cAMP levels in cells growing under different conditions are not precisely known. However, a variety of genetic^{14–16} and biochemical²⁶ experiments suggest that cells growing in glucose probably have higher cAMP levels than cells growing in poorer nutrients. This high cAMP may partially repress *CLN1* and other genes, and cause a larger critical cell size.

What is the adaptive significance of the control of Cln expression by cAMP and nutritional cues? The Cln proteins have

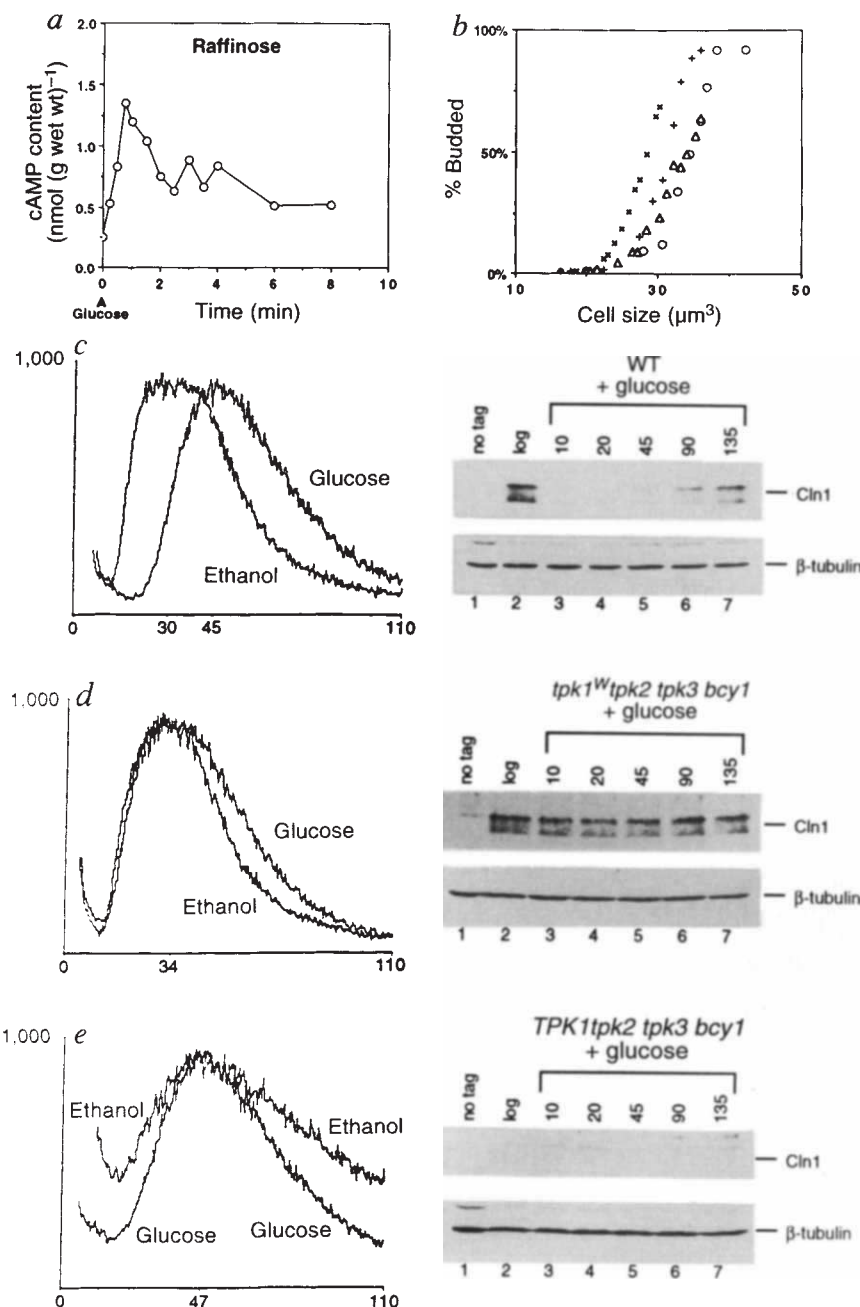
extremely short half-lives^{27,28} and therefore exist in an equilibrium, with synthesis balanced against turnover. At low protein synthesis rates, the maximal level of Cln is necessarily low. The cAMP pathway may help a slowly growing cell acquire sufficient G1 cyclin for Start by balancing *CLN* expression against protein synthesis. When protein synthesis is slow, low cellular cAMP permits relatively high levels of *CLN* expression (that is, higher levels than in the absence of this balancing system, though not

higher absolute levels). When synthesis is rapid, high cellular cAMP reduces *CLN* expression, helping balance growth with division. Slowly growing cells have lower absolute levels of *CLN* mRNAs and proteins than rapidly growing cells (G.T., B. Schneider and B.F., unpublished results), so this cAMP 'balancing' mechanism does not keep Cln levels constant at all growth rates—it is simply a helpful force in that direction.

This speculative idea predicts that cells lacking the balancing

FIG. 2 Glucose fails to regulate Cln1 or cell size in cells lacking the Ras/cAMP signalling pathway. **a**, Increase in intracellular cAMP after addition of glucose to cells growing in YEP + 2% raffinose. **b**, *cln1* deletion strains are partly defective in nutrient regulation of size. Samples of small, unbudded *CLN1* or *cln1* cells of uniform size were grown in glucose or ethanol medium, and the percentage of budded cells was assayed as a function of cell size. $\times$, *CLN1* cells growing in YEP + 2% ethanol; $+$, *CLN1* cells growing in YEP + 2% ethanol and 2% glucose; Δ , *cln1* cells growing in YEP + 2% ethanol; $\circ$, *cln1* cells growing in YEP + 2% ethanol + 2% glucose. Critical sizes for budding (that is, the mode cell volume when 50% of the cells had buds) were: *CLN1*, ethanol, 28.5 μm^3 ; glucose, 31.2 μm^3 ; *cln1*, ethanol, 34.1 μm^3 ; glucose, 34.7 μm^3 . **c** (left), Cell size distributions for wild-type cells growing in glucose or ethanol medium. The approximate modes of the two size distributions are shown in μm^3 . 1,000 cells were assayed in the peak channel. **c**, right, Immunoblot showing repression of Cln1 protein. Glucose was added to wild-type cells carrying *CLN1C* growing in raffinose, and samples were taken at the times indicated (min). 'No tag' and 'log' controls are as in Fig. 1. Cln1 protein and β -tubulin (loading control) were measured. **d**, As **c**, but the strain was *tpk1^w tpk2 tpk3 bcy1* (a 'wimp' strain). **e**, As **c** and **d**, but the strain was *TPK1 tpk2 tpk3 bcy1* (that is, with unregulated Tpk1), and twice as much protein was loaded.

METHODS. All strains were isogenic with W303. **a**, Cyclic AMP levels were determined as described³⁰ using the cAMP ³H-assay kit from Amersham. **b**, For analysis of critical size, isogenic *CLN1* or *cln1* cells were grown to mid-log phase in YEP + 2% ethanol at 30 °C. Unbudded cells with a mode volume of 20 μm^3 were isolated by centrifugal elutriation at 23 °C. Cells were reinoculated into the same medium, and also into the same medium supplemented with 2% glucose, and grown at 25 °C. Samples were taken at 15-min intervals for 255 min, and cell volumes were assayed with the Coulter Channelyzer. Per cent budding was assayed using coded sample tubes. For each of the four experiments, the two samples with just less than 50% budding, and the two samples with just more than 50% budding, were analysed in greater detail. For these samples, blind counts of percentage budding were done in triplicate on a total of more than 700 cells per sample. Critical size for budding (here defined as the mode volume of the cell fraction when 50% of the cells had buds) was calculated from the best-fit line using these four points. For **d**, a wimp strain was created in the W303 background by disrupting *TPK2*, *TPK3* and *BCY1* with *HIS3*, *TRP1* and *LEU2*, respectively. Disruption plasmids were obtained from L. Rodgers and M. Wigler; disruptions were checked by Southern analysis. The resulting strain was extremely sensitive to heat shock. Heat-shock-resistant *tpk1^w* revertants were obtained and checked by genetic analysis. For cell size analysis, wild-type and wimp strains were grown to mid-log phase in YEP + 2% glucose, or YEP + 2% ethanol, sonicated,



and assayed with a Coulter Channelyzer. The same medium was used for the *TPK1 tpk2 tpk3 bcy1* strain, except that it was supplemented with 1 M sorbitol (required for growth of this strain in YEP ethanol). The sorbitol reduces cell volumes slightly. Generation times for the various cultures were: wild type in glucose, 85 min; in ethanol, 145 min; wimp strain in glucose, 95 min; in ethanol, 145 min; *TPK1 tpk2 tpk3 bcy1* strain in glucose, 85 min; in ethanol, 200 min. For immunoblot analysis, the same strains were transformed with plasmid pCLN1 IV, which carries *CLN1* tagged at the 3' end with a triple copy of the HA epitope in the *URA3 CEN* plasmid YCp50. Immunoblots were carried out as in Fig. 1.

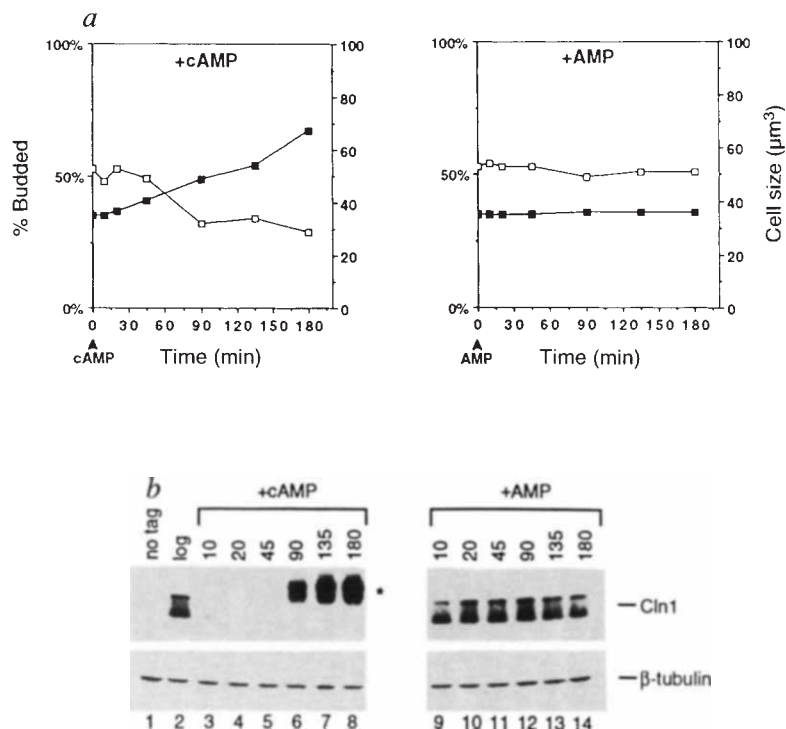


FIG. 3 cAMP causes a pause in G1 and represses *CLN1*. **a**, Cell volumes (filled squares) and the percentage of budded cells (open squares) were assayed for a *pde2* strain after addition of cAMP (left) or 3' AMP (right) to 5 mM. **b**, Immunoblot showing repression of Cln1 protein after addition of cAMP (left) to a culture of *pde2* cells. The bands seen in the cAMP experiment in lanes 6–8 (asterisk) are not Cln1, because they are also seen in an untagged control strain (data not shown) and because they appear at a time when no *CLN1* mRNA is present (Fig. 4). They are due to some other protein induced by cAMP. The same blot was reprobed for β -tubulin as a loading control.

METHODS. A *pde2::TRP1* strain isogenic with W303 and containing a triple HA-tagged *CLN1* allele²⁹ was grown in YEP + 2% raffinose to a density of 1×10^7 cells per ml, at which time a control sample was removed. The remaining culture was split into two, then either cAMP or 3' AMP was added to 5 mM and samples were taken as indicated. Samples were analysed for protein, mRNA and budding index as described²⁹. Cell volumes are the mode of the distribution determined with a Coulter Channelyzer. The *pde2::TRP1* disrupting plasmid was provided by K. Tatchell.

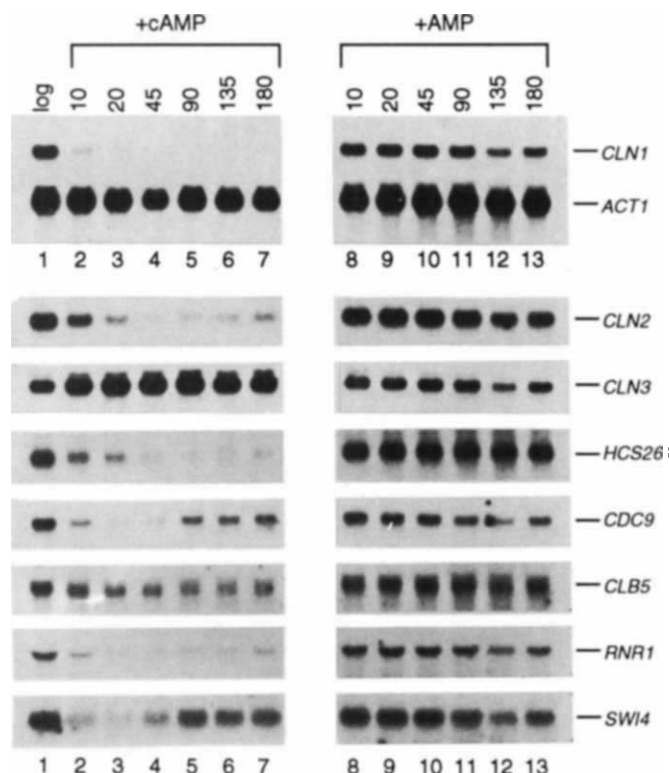


FIG. 4 High levels of cAMP repress many SBF- and MBF-regulated genes. Either cAMP or 3'-AMP was added to W303 *pde2::TRP1* cells growing in raffinose, and samples were analysed for mRNA by northern blotting. Lane 1, total RNA from the culture before addition of cAMP or 3'-AMP. Remaining lanes show samples taken at the indicated times (min) after addition of cAMP (lanes 2–7) or 3'-AMP (lanes 8–13).

METHODS. Northern analysis was done as for Fig. 1 but with probes for *CLN1*, *ACT1*, *CLN2*, *CLN3*, *HCS26*, *CDC9*, *CLB5*, *RNR1* and *SWI4* (refs 20, 29). Of these, *CLN1*, *CLN2* and *HCS26* are SBF-regulated, and *CDC9*, *RNR1* and *CLB5* are MBF-regulated. *SWI4* is also probably regulated by SBF or MBF.

mechanism might become large at low growth rates, where steady-state Cln levels are low. This may be true of wimp strains in glycerol medium (data not shown). A hint of such an effect in wild-type cells—a slightly increased size at extremely low growth rates—can be seen in data from early experiments on growth rate and cell size^{2,3}. □

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Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE

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RECENT studies suggest that proteases of the interleukin 1- β -converting enzyme (ICE)/*ced-3* family are involved in initiating the active phase of apoptosis¹⁻³. Here we identify a novel protease resembling ICE (prICE) that is active in a cell-free system that reproduces the morphological and biochemical events of apoptosis⁴. prICE cleaves the nuclear enzyme poly(ADP-ribose) polymerase (PARP) at a tetrapeptide sequence identical to one of two ICE sites in pro-interleukin-1- β . However, prICE does not cleave purified pro-interleukin-1- β , and purified ICE does not cleave PARP, indicating that the two activities are distinct. Inhibition of prICE abolishes all manifestations of apoptosis in the extracts including morphological changes, cleavage of PARP and production of an oligonucleosomal ladder. These studies suggest that prICE might be pivotal in initiating the active phase of apoptosis *in vitro* and in intact cells.

To study apoptotic events, we have developed a cell-free system that uses cytoplasmic extracts made from chicken DU249 cells that become committed to apoptosis as a result of an S-phase aphidicolin arrest and are subsequently collected in M phase (S/M extracts⁴). Cleavage of PARP to a fragment of relative molecular mass $\sim 85,000$ ($M_r \sim 85K$), an early event in apoptosis in intact cells⁵, was observed within three minutes after addition of isolated nuclei or purified PARP to S/M extracts (Fig. 1a). The PARP fragments produced *in vivo* and *in vitro* were indistinguishable by SDS-PAGE (Fig. 1b). Control, real mitotic extracts (RME) prepared without aphidicolin presynchronization did not induce apoptotic changes in added nuclei⁴ and did not cleave PARP (Fig. 1b).

Proteolysis appeared to be highly selective. Immunoblotting revealed that several other nuclear proteins, including topoisomerase II (170K), ScII (135K⁶), CENP-C (140K⁷) and RCC1 (45K⁸, Fig. 1a) remained intact for at least 1 h in S/M extracts. In addition, the mobilities of major proteins visible on stained SDS gels were unaltered by incubation of nuclei in S/M extracts (data not shown).

Amino-terminal sequencing of the purified 85K PARP fragment revealed that cleavage occurred between Asp 216 and Gly 217 (data not shown), a site that is conserved in human⁹, bovine¹⁰ and chicken¹¹ PARP. The region immediately surrounding the cleavage site in PARP (Glu-Val-Asp.../...Gly) is identical to one of the two ICE cleavage sites in pro-interleukin-1- β . A decamer peptide spanning the putative cleavage site inhibited PARP cleavage, whereas a peptide containing Ala in place of Asp at the P₁ position failed to inhibit PARP cleavage (Fig. 1c). Thus Asp in the P₁ position is essential for catalytic recognition by the PARP protease. Only two eukaryotic proteases are known to cleave next to Asp: granzyme B¹², a serine protease that initiates cytotoxic lymphocyte-induced apoptosis^{13,14}; and ICE^{15,16}. Inhibitor studies (not shown) indicated that the PARP protease was sensitive to iodoacetamide but resistant to the cysteine protease inhibitor E-64 and the serine protease inhibitors PMSF, TLCK or TPCK, a pattern that matches the inhibitor profile of ICE¹⁷. These results suggest that PARP cleavage involves a protease resembling ICE (prICE).

To explore the possibility that prICE is ICE itself, we examined the stability of human pro-interleukin-1- β in S/M extracts. Pro-interleukin-1- β (31K) was cleaved by purified ICE but not by S/M extracts (Fig. 2a, lower). Conversely, purified PARP was cleaved by S/M extracts but not by ICE (Fig. 2a, upper).

Because prICE cleaves PARP at an ICE-like cleavage site, we examined the effect of a specific tetrapeptide ICE inhibitor on apoptotic events in S/M extracts. When YVAD chloromethylketone¹⁶ was added to S/M extracts before nuclei, concentrations as low as 4 μM blocked all apoptotic events including PARP cleavage (Fig. 2b), morphological changes (Fig. 2c) and internucleosomal DNA fragmentation (data not shown). Iodoacetamide had a similar effect. In controls, TPCK, another chloromethylketone, had no effect at concentrations as high as 140 μM (Fig. 2b, c).

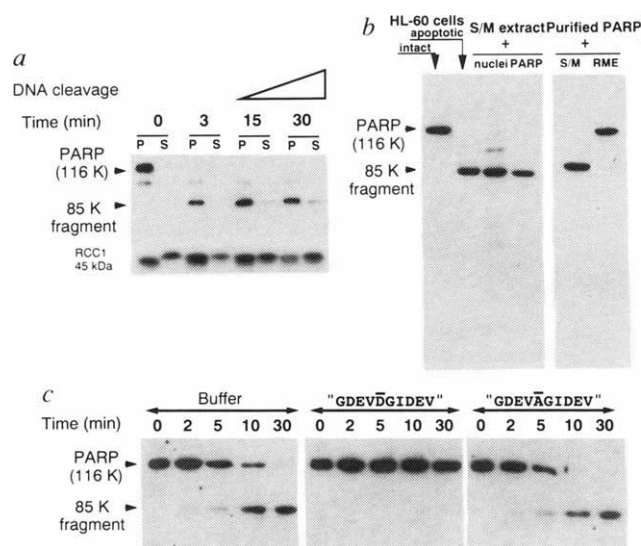
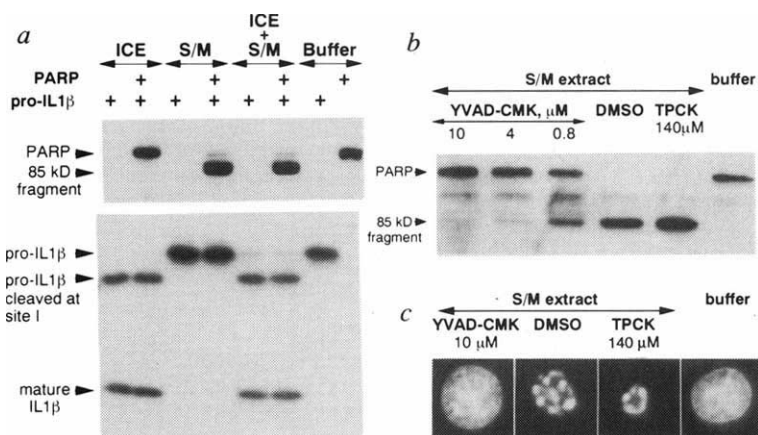


FIG. 1 PARP is cleaved at a site identical to one of the ICE cleavage sites in pro-interleukin-1- β . a, Time course of cleavage of PARP during apoptosis *in vitro*. S/M extract was diluted with DB to a final protein concentration of 10 mg ml⁻¹. Isolated HeLa nuclei were added at a concentration of 40,000 per μ l. After incubation at 37 °C for the indicated time, the nuclei (pellet, P) were separated from the extract (supernatant, S) by centrifugation at 12,000g for 5 min, and both were subjected to electrophoresis followed by immunoblotting with monoclonal antibody C-2-10 as previously described⁵. This antibody recognizes an epitope located between the zinc fingers and automodification domains of PARP^{5,21}. The band between the 116K and 85K forms of PARP represents a cross-reacting species present in some preparations of nuclei. b, Apoptotic cleavage of PARP produces fragments of the same size in intact HL-60 cells and *in vitro* in S/M extracts and shows that PARP is cleaved only in extracts that have apoptotic activity. HL-60 cells were treated with 17 μM etoposide for 4 h to induce apoptosis⁵. HeLa nuclei and purified bovine PARP²² were incubated with S/M extract. Right two lanes, 200 ng of purified PARP were incubated with 10 μ l of either S/M extracts or RME for 30 min at 37 °C. All samples were subjected to SDS-PAGE followed by immunoblotting with antibody C-2-10. c, Inhibition of prICE activity by a decamer peptide spanning the PARP cleavage site: Asp at the P₁ position is essential for inhibition of prICE. Peptides GDEVDGIDEV or GDEVAGIDEV were dissolved in 50 mM PIPES, pH 7.4, at a final concentration of 5 mM. Purified bovine PARP was dialysed against DB and diluted with this buffer to 10 μ g ml⁻¹. PARP (45 μ l) was mixed with 15 μ l of either one of the peptides or with 50 mM PIPES. S/M extract (4 μ l) was added to each of the samples (final protein concentration 2 mg ml⁻¹), which were then incubated at 37 °C. At the indicated times, an aliquot of each sample was mixed with an aliquot of SDS sample buffer and heated for 3 min at 95 °C. The resulting samples were subjected to SDS-PAGE and immunoblotting with antibody C-2-10. Peptide GDEVDGIDEV inhibited cleavage of PARP by the apoptotic extract under these conditions, whereas peptide GDEVAGIDEV did not. A similar D $\rightarrow$ A change in pre-IL-1 β abolishes cleavage of the precursor by ICE¹⁵.

FIG. 2 a, prICE does not cleave human pro-interleukin-1 β (pro-IL-1 β), and human ICE does not cleave bovine PARP. Recombinant human pro-IL-1 β was purchased from Cistron Biotechnology (Pine Brook, NJ) as a 10 $\mu\text{g ml}^{-1}$ solution in 10 mM Tris, 0.1% Triton X-100, 0.1 mM EDTA and 10% glycerol. Aliquots (8 μl) of ICE assay buffer (25 mM HEPES, 5 mM DTT, 10% glycerol¹⁵) were supplemented with 1 μl (10 ng) of pro-IL-1 β solution in DB or in DB plus 1 μl (150 ng) of bovine PARP. In the samples that contained both PARP and pro-IL-1 β , the molar ratio of PARP to pro-IL-1 β was about 4:1. Next, each sample was supplemented with 1 μl (1.5 U) of ICE, 1 μl (45 μg of total protein) of an S/M extract, or both. Samples were then incubated at 37 °C for 30 min, mixed with an aliquot of SDS sample buffer and heated for 3 min at 95 °C. The resulting samples were subjected to electrophoresis in 15% polyacrylamide gels followed by immunoblotting. The blot was probed with a polyclonal antibody that recognizes both the precursor and mature IL-1 β (Cistron Biotechnologies), and then with ¹²⁵I protein A. After autoradiography, the same blot was probed with murine anti-PARP antibody C-2-10 followed by peroxidase-labelled anti-mouse and detected by ECL. b, c, Inhibition of prICE by YVAD·chloromethylketone blocks all apoptotic events in S/M extracts. b, YVAD·CMK, (Ac-YVAD·chloromethylketone) a specific inhibitor of ICE, inhibits prICE. A 20 mM stock solution of YVAD·CMK (Bachem), was diluted with 50 mM PIPES, pH 7.4 and then added to aliquots of an S/M extract at the indicated final concentrations. Control aliquots were supplemented with 0.1% DMSO to evaluate the effect of the solvent, or with 140 μM of tosylphenylalanyl-chloromethylketone (TPCK) to exclude possible non-



specific effects of the chloromethylketone moiety. After aliquots were preincubated for 15 min at 37 °C, HeLa nuclei were added at a concentration of 100,000 per μl , incubated for 1 h at 37 °C, and the samples were subsequently subjected to SDS-PAGE and immunoblotting for detection of PARP. c, YVAD·CMK inhibits apoptotic changes *in vitro*. HeLa nuclei were incubated for 1 h at 37 °C in aliquots of S/M extract that had been preincubated with the indicated concentrations of YVAD·CMK, DMSO or TPCK as described above. An aliquot of nuclei from each sample was stained with DAPI to reveal changes in nuclear morphology.

In summary, we have shown that extracts from cells committed to apoptosis contain a protease activity (prICE) that shares many features with ICE including (1) cleavage at the same tetrapeptide sequence, (2) sensitivity to proteinase inhibitors and (3) inhibition by the specific chloromethylketone YVAD·CMK. prICE is, however, distinguished from ICE by its substrate specificity. Although it is formally possible that chicken ICE does not cleave mammalian pro-interleukin-1 β , we note that purified mammalian ICE failed to cleave purified mammalian PARP under all conditions tested. As PARP is cleaved during apoptosis in mammalian cells⁵, this clearly implicates the involvement of a second ICE-like enzyme in apoptosis.

Although PARP cleavage might serve an essential function in apoptosis, by decreasing consumption of NAD⁵ and its precursor ATP, an energy source that is required for completion of apoptosis *in vivo*^{18,19} and in S/M extracts (Y.L. and W.C.E., unpublished), it is unlikely that PARP cleavage is the only function of prICE. Other substrates cleaved by prICE during the initiation of apoptosis (possibly including nucleases and additional proteases) might be activated and go on to trigger the morphological events of apoptosis. The observation that YVAD·CMK prevents the biochemical and morphological changes of apoptosis in S/M extracts raises the possibility that prICE may lie near the apex of an apoptotic cascade both *in vivo* and *in vitro*. □

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Interleukin-2 transcriptional block by multifunctional Ca²⁺/calmodulin kinase

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IN the presence of costimulation¹, Ca²⁺ influx in T cells leads to activation (transcription of interleukin-2; ref. 2) via calcineurin^{3,4}. In the absence of costimulation, Ca²⁺ influx results in anergy (interleukin-2 transcriptional block⁵) through an unknown mechanism. Specific attenuation of interleukin-2 transcriptional induction occurs in Jurkat T cells following pretreatment with a Ca²⁺ ionophore. A >90% block of inducible interleukin-2 reporter gene activity was initiated by transfection of a constitutively active mutant of multifunctional Ca²⁺/calmodulin-dependent protein kinase (CaM kinase or CaM kinase II)⁶, but not by constitutive mutants

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of CaM kinase IV, calcineurin or protein kinase C. The block was complete six hours after kinase transfection and showed specificity for interleukin-2; there was no change in β -actin transcription or in *c-fos* transcription induced by phorbol myristyl acetate, and a Rous sarcoma virus promoter was stimulated threefold. Multifunctional CaM kinase also attenuated interleukin-2 activation by calcineurin plus phorbol ester. T-cell receptor signalling activates multifunctional CaM kinase. These findings suggest that two Ca^{2+} /calmodulin-responsive enzymes, multifunctional CaM kinase and calcineurin, could mediate the divergent effects of Ca^{2+} signals in T-lymphocyte regulation.

We examined whether multifunctional (type II) CaM kinase⁶ is activated during stimulation of intact Jurkat T cells by monitoring its conversion from a Ca^{2+} -dependent to a partially Ca^{2+} -independent enzyme⁷. Indeed, both Ca^{2+} ionophore and T-cell receptor (TCR) monoclonal antibody generated autonomous CaM kinase activity (Fig. 1b). This indicates that activation and autophosphorylation of the kinase occurred in the cells before lysis and kinase assay. Surprisingly, TCR stimulation was more effective in activating CaM kinase, despite the fact that ionophore produced a larger increase in the intracellular calcium concentration ($[\text{Ca}^{2+}]_i$, Fig. 1a). A possible explanation for this is that CaM kinase is more responsive to oscillations in $[\text{Ca}^{2+}]_i$, such as those produced by T-cell receptor activation⁸, than to the stable elevation produced by ionomycin^{9,10}. Although the fraction of Ca^{2+} -independent (autonomous) kinase molecules diminished within 10 min of stimulation, the kinase is likely to be active as long as $[\text{Ca}^{2+}]_i$ is elevated (Fig. 1 legend). Activation of CaM kinase was insensitive to the calcineurin inhibitor cyclosporin A (Fig. 1b).

The interleukin-2 enhancer has been shown to reflect accurately negative¹¹ as well as positive¹² transcriptional regulation. We therefore used this enhancer linked to a reporter gene to examine whether negative regulation of interleukin-2 occurs in Jurkat T cells. Pretreatment of the cells with Ca^{2+} ionophore for 6–12 h in the absence of a costimulatory signal, a treatment which induces interleukin-2 transcriptional block in mature T-cell clones⁵, diminished subsequent induction of the interleukin-2 reporter gene by Ca^{2+} ionophore and phorbol myristyl acetate (PMA) by >50% (Fig. 2a). This ionophore-mediated interleukin-2 attenuation was specific (Fig. 2 legend). The half-life of

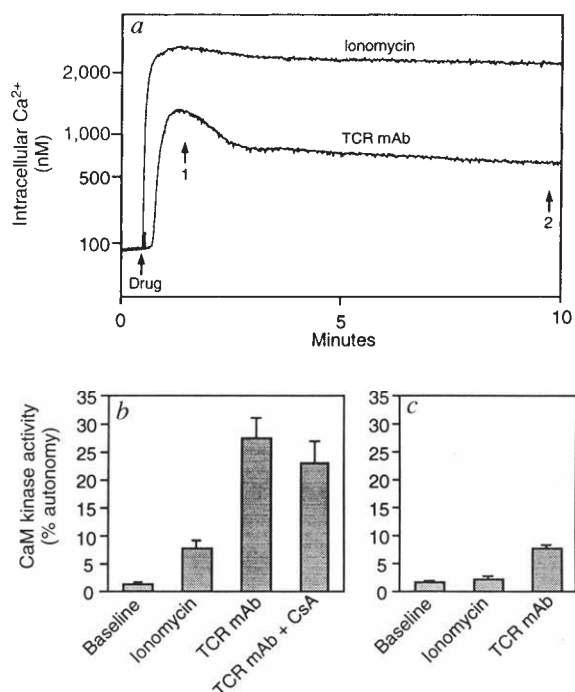
the interleukin-2 block in Jurkat cells was 12 h, substantially shorter than that in T-cell clones. This may be due to rapid cell division in Jurkat T cells, as cell cycling reverses interleukin-2 transcriptional block in T-cell clones¹.

We tested several potential mediators of this Ca^{2+} -induced interleukin-2 unresponsiveness in Jurkat T cells. Although calcineurin has been suggested to be a mediator of both activation and anergy in T cells, cells transfected with constitutive calcineurin³ and incubated in the absence of costimulation showed no block of subsequent interleukin-2 reporter gene induction by PMA and ionomycin, but instead showed a threefold increase over controls (Fig. 2a). We have cloned a human T-cell CaM kinase, γ_B -CaM kinase¹³, and found that a recessively inherited susceptibility to autoimmune myocardial disease, *amd*, maps to a region of murine chromosome 14 which is likely to be near γ -CaM kinase^{14,15}. By mutating a single nucleotide in its autoinhibitory domain, we generated γ_B -CaM kinase (c), which had 37% activity in the absence of Ca^{2+} -calmodulin¹³. Importantly, the substrate specificity of γ_B -CaM kinase was not detectably altered by this mutation (Fig. 2 legend). Indeed, transfection of γ_B -CaM kinase (c) blocked interleukin-2 reporter gene induction by PMA and ionomycin by >90% (Fig. 2a). If overnight stimulation was begun only 10 min after γ_B -CaM kinase (c) transfection, the interleukin-2 block was 59%; after 4 h it was 85% and after 6–72 h >90% of inducible activity was blocked. The 10-min time point suggests that significant CaM kinase expression occurred before interleukin-2 transcriptional commitment, which requires 3–6 h. Because calmodulin is required for calcineurin activation, it is possible that γ_B -CaM kinase (c) expression affected interleukin-2 activation indirectly by binding calmodulin and thereby inhibiting calcineurin activity. To control for this possibility, we prepared a mutant T-cell CaM kinase, γ_B -CaM kinase (i), which was catalytically inactive yet retained normal calmodulin-binding properties. Neither this inactive mutant nor a double inactive/constitutive mutant had any effect on interleukin-2 activation (Figs 2a and 3).

We tested two other constitutive protein kinases for their ability to affect interleukin-2 transcriptional induction. Constitutive protein kinase C¹⁶ had no effect on interleukin-2 stimulation by PMA and ionomycin (Fig. 2a), but caused 30% activation in the presence of ionomycin alone. CaM kinase IV (CaM kinase-

FIG. 1 Multifunctional CaM kinase is activated in Jurkat T cells by signals from the T-cell receptor. a, Intracellular Ca^{2+} traces of stimulated Jurkat T cells. Ionomycin (2 μM) or anti-T-cell receptor monoclonal antibody C305 (gift of A. Weiss, used at 1:1,000) were added at the arrow labelled 'Drug'. Arrows labelled 1 and 2 indicate time points at which kinase assays were carried out in b (1 min) and c (10 min), respectively. b, CaM kinase autonomy 1 min after stimulus. Extent of conversion of CaM kinase to a Ca^{2+} -independent or autonomous species was assessed by *in vitro* kinase assays in the absence and presence of Ca^{2+} -calmodulin. Cells treated with cyclosporin A (+CsA) were preincubated with 100 ng ml^{-1} cyclosporin A for 15 min before stimulation for 60 s. c, CaM kinase autonomy 10 min after stimulus. Autonomy (Ca^{2+} -independent activity) is reversed by dephosphorylation in the regulatory region of the kinase¹⁸ which occurs quite rapidly after activation. However, Ca^{2+} -stimulated CaM kinase activity (assayed in the presence of Ca^{2+} /calmodulin) was no less at 10 min than at 1 min (data not shown). Thus, the kinase is likely to be active much longer than it is autonomous, essentially while $[\text{Ca}^{2+}]_i$ remains elevated.

METHODS. Jurkat T cells obtained from the ATCC were loaded with Indo-1 (Molecular Probes) and analysed at 37 °C in an SLM 8000 spectrofluorimeter with excitation at 360 nm and the ratio of Ca^{2+} -bound dye (405 nm) to Ca^{2+} -free dye (485 nm) was recorded. Calibration was as described²⁷. Following stimulation, kinase assays were carried out on a whole-cell extract of 10^7 Jurkat T cells using the synthetic peptide autocamtide-2 which is specific for CaM kinase II over CaM kinase IV and other kinases as described^{7,13}. Autonomy is defined as the Ca^{2+} -independent CaM kinase activity expressed as a percentage of maximal Ca^{2+} -stimulated activity. Duplicate kinase assays were done in each experiment and results are the mean ($\pm$ s.e.m.) of 3–8 separate experiments.



Gr) phosphorylates the same minimal consensus substrate sequence as multifunctional CaM kinase, Arg-Xxx-Xxx-Ser/Thr^{17,18}, and has been shown to be activated through T-cell receptor signalling¹⁹. Although transfection of constitutive CaM kinase IV generated significant Ca²⁺-independent CaM kinase IV activity, this kinase had no effect on interleukin-2 induction (Fig. 2a).

Analysis of the critical transcriptional regulatory sites in the interleukin-2 enhancer^{2,12} revealed that γ_B -CaM kinase (c) blocked 37–69% of stimulated transcriptional activity from multimers of NFAT, AP-1, and NFIL-2A DNA elements (Fig. 2b). The effect of CaM kinase on these individual regions was additive, blocking >90% of interleukin-2 transcription from the endogenous enhancer. Interestingly, CaM kinase significantly decreased stimulated transcription from AP-1, which has been found to be blocked following induction of anergy in T-cell clones¹¹.

We used three control promoters to test whether the γ_B -CaM kinase (c) block of interleukin-2 transcription could be due to

nonspecific or toxic effects. γ_B -CaM kinase (c) did not affect β -actin-chloramphenicol acetyltransferase (CAT) transcription or the ability of PMA to double *c-fos* transcription (Fig. 2b). As expected²⁰, it stimulated Rous sarcoma virus (RSV) promoter threefold. Variability in transfection efficiency was eliminated by simultaneously transfecting kinase constructs with β -galactosidase, luciferase and CAT reporter constructs. Figure 3 shows a single experiment in which the expression levels of γ_B -CaM kinase constructs revealed by calmodulin blotting are correlated with interleukin-2, *c-fos* and RSV reporter gene activities.

Can CaM kinase inhibit the stimulatory pathway mediated by calcineurin and protein kinase C? Indeed, γ_B -CaM kinase (c) blocked the ability of constitutive calcineurin plus PMA to activate interleukin-2 (Fig. 4). It is unlikely that this antagonism involves a direct interaction between kinase and phosphatase, as neither phosphorylation of calcineurin in the calmodulin-binding site nor possible dephosphorylation of CaM kinase by calcineurin would occur with constitutive constructs. Instead, CaM kinase may antagonize the action of calcineurin by phos-

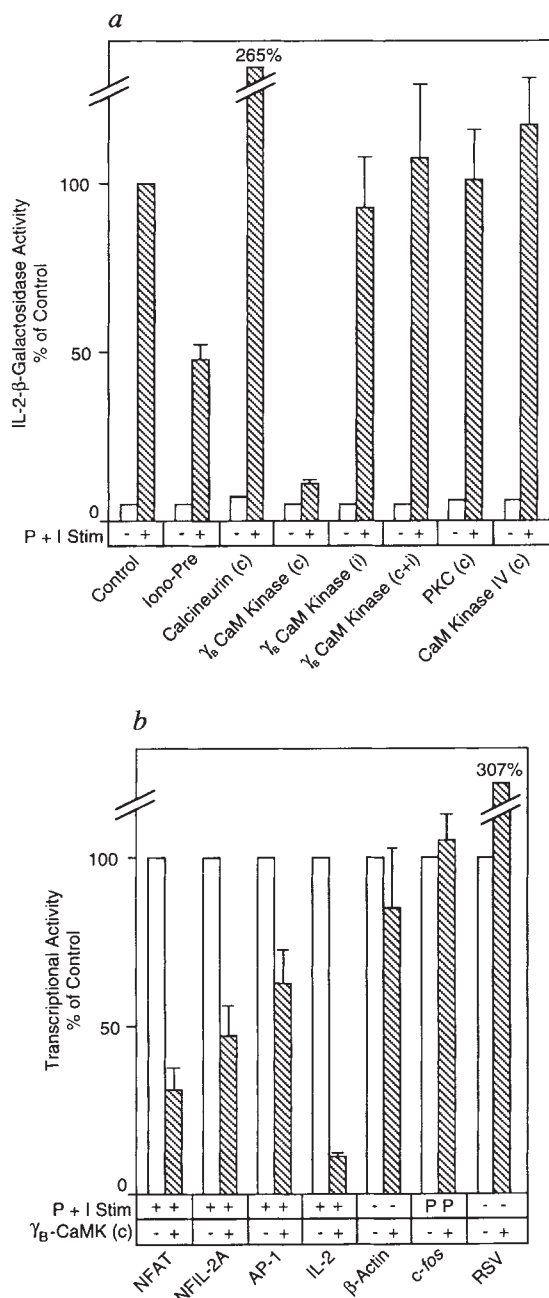


FIG. 2 Characterization of interleukin-2 (IL-2) transcriptional block by multifunctional CaM kinase. **a**, Effect of various treatments of Jurkat T cells on subsequent IL-2 β -galactosidase transcriptional activation by PMA plus ionomycin (P + I). Cells were cotransfected with IL-2 (ref. 28) and *c-fos*²⁹ reporter constructs and one of the following test constructs: Control and Iono-Pre, the SR α expression vector³⁰; calcineurin (c), constitutive calcineurin construct Δ CaM-AI³¹; γ_B -CaM kinase (c), constitutive γ_B -CaM kinase¹³; γ_B -CaM kinase (i), inactive γ_B -CaM kinase; γ_B -CaM kinase (c + i), double mutant constitutive/inactive; PKC (c), constitutive protein kinase C¹⁶; CaM kinase IV (c), constitutive CaM kinase IV¹⁷. Iono-Pre cells were treated with 2 μ M ionomycin for 8 h before overnight stimulation. Attenuation of IL-2 transcription by ionophore pretreatment seemed to be specific as it did not block transcription from the human *c-fos* promoter ($106 \pm 9\%$ of control) or a RSV promoter²⁰ ($121 \pm 13\%$ of control). Results are means ($\pm$ s.e.m.) of 5–15 independent experiments, normalized to control transfection assayed in parallel. **b**, Constitutive γ_B -CaM kinase affects multiple IL-2 enhancer sites but does not block other promoters. Jurkat T cells were transfected with a reporter construct (NFAT²⁸, NFIL-2A²⁸, AP-1²⁸, IL-2²⁸, β -actin²⁹, *c-fos*²⁹ or RSV²⁰) and either SR α as control (open bars, —, defined as 100%) or γ_B -CaM kinase (c) (shaded bars, +), then stimulated overnight with PMA + ionomycin (+), stimulated with PMA only (P) or left unstimulated (–) as indicated. All controls were done in parallel. Results are mean $\pm$ s.e.m. for 3–15 independent experiments.

METHODS. After transfection of 10^7 Jurkat T cells (strain with optimal IL-2 induction; gift from G. Crabtree) by electroporation as described^{3,13}, cells were incubated for 48 h unless otherwise specified, then either not treated or stimulated overnight as indicated with PMA (50 ng ml⁻¹) and ionomycin (2 μ M) before collection. We detected no alteration in the substrate specificity of the constitutive γ_B -CaM kinase mutant compared with the wild type in assays with a panel of peptide substrates specific for six protein kinases: protein kinase A, protein kinase C (both α and ϵ isoforms), and three distinct classes of CaM kinase (types I, II (multifunctional) and IV). The inactive γ_B -CaM kinase was constructed by site-directed mutagenesis of the lysine at position 43 to methionine in the highly conserved ATP-binding domain of γ_B -CaM kinase. *In vitro*, this catalytically disabled mutant retained undetectable (<2% of wild-type γ_B -CaM kinase) activity on stimulation with Ca²⁺/calmodulin. The constitutive CaM kinase IV construct pVLC-CaMKIV¹⁷ was digested with Not I-ApaI, then subcloned into SR α at the EcoRV-ApaI site (by E. K. Heist). The SR α expression vector³⁰ was used for all six expression constructs in this study. β -Galactosidase²⁸, CAT¹² and luciferase¹² were assayed as described.

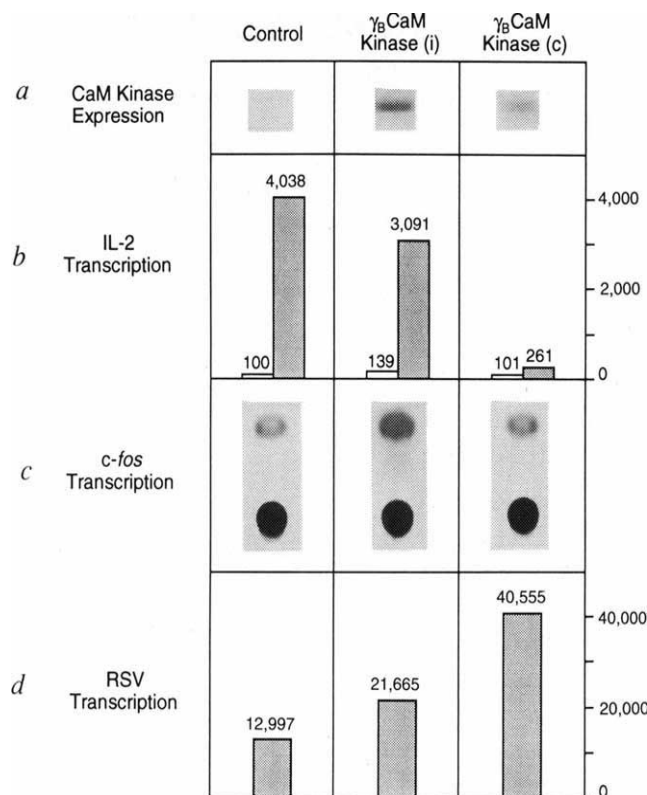


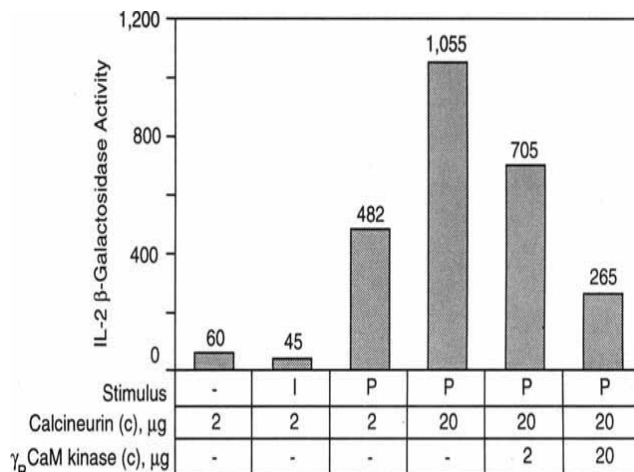
FIG. 4 Constitutive T-cell CaM kinase attenuates interleukin-2 activation by constitutive calcineurin plus PMA. Jurkat T cells were cotransfected with the IL-2 reporter construct and the indicated amount of constitutive calcineurin and constitutive CaM kinase as described for Fig. 2. The expression level of CaM kinase protein increased approximately 10-fold by transfection of 20 versus 2 μ g of the constitutive construct (not shown). Total DNA added in each transfection was adjusted to be constant by the addition of SR α expression vector DNA where required. After 48 h of incubation, the indicated drug was added for 12–16 h before collection and β -galactosidase assay. Results are the mean of triplicate β -galactosidase assays and representative of four separate experiments.

phorylating critical transcription factors such as NF-AT β , a component of NF-AT which may be dephosphorylated by calcineurin²¹ and contains 12 consensus phosphorylation sites for CaM kinase²².

Although these data in Jurkat T cells suggest involvement of CaM kinase in a Ca²⁺-dependent interleukin-2 transcriptional block reminiscent of T-cell clonal anergy, definitive proof will require evidence such as the loss of anergy following knockout or inhibition of multifunctional CaM kinase. Costimulatory signals¹ could determine the outcome of antagonism between CaM kinase phosphorylation and calcineurin dephosphorylation. By analogy, the frequency of neuronal stimulation determines the outcome of antagonism²³ between CaM kinase activation, which leads to synaptic potentiation^{24,25}, and calcineurin activation, which leads to synaptic depression²⁶. Further characterization of Ca²⁺-responsive enzymes involved in lymphocyte regulation should clarify the molecular mechanisms of self-tolerance as well as suggest novel targets for immunoregulation. □

FIG. 3 CaM kinase expression levels and transcriptional effects in single populations of transfected cells. **a**, The level of multifunctional CaM kinase expression is revealed by SDS-gel electrophoresis and calmodulin blot of cytosolic protein from transfected Jurkat T cells. Cells were transfected with SR α (Control), inactive CaM kinase (γ_B -CaM kinase (i)) or constitutive CaM kinase (γ_B -CaM kinase (c)), together with reporter genes as described below. **b**, Interleukin-2 transcription as assessed by the IL-2 enhancer- β -galactosidase reporter construct, IL2ZH. Aliquots of transfected cells were incubated for 48 h, then either not stimulated (open bars) or stimulated with PMA plus ionomycin (shaded bars) before collection. **c**, c-fos transcription as assessed by a CAT reporter gene. Following cotransfection with pFC700 (ref. 29), cells were incubated for 48 h then stimulated overnight with PMA before collection and assay. **d**, Rous sarcoma virus promoter-mediated transcriptional activity in cells cotransfected with the RSV-luciferase construct. Cells were collected following 60 h of incubation without stimulation.

METHODS. Results shown in **a–d** were derived from a single experiment in which three populations of Jurkat T cells were transfected as in Fig. 2 with either SR α (Control) or the indicated CaM kinase construct and the IL-2, c-fos and RSV reporter genes. Assays for β -galactosidase²⁸, CAT¹², luciferase¹², and quantification of kinase protein based on calmodulin overlay on nitrocellulose¹³ were as described. CAT activity was quantified by autoradiography and laser densitometry (Molecular Dynamics).



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Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology

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INSULIN-DEPENDENT diabetes mellitus (IDDM) is a T-cell-mediated autoimmune disease whose onset is believed to be triggered by unknown environmental factors acting on a predisposing genetic background. Islet-infiltrating T (IIT) cells from two IDDM patients, who had died at the onset of the disease from brain swelling as a complication of ketoacidosis, were analysed. The results provided evidence for the involvement of a pancreatic islet cell membrane-bound superantigen^{1,2} as a diabetes aetiopathogenetic factor. There was a selective expansion of a T-cell receptor (TCR) variable segment of the β -chain (V β 7) in these IIT cells in association with unselected V α -chain segments; extensive junctional diversity of the TCR V β 7 chains; and evidence of positive selection, after exposure to diabetic islet cell membrane preparations, of V β 7⁺ T-cell clones among peripheral blood lymphocytes from non-diabetic individuals.

In the past two years we have been able to obtain and study the pancreas and spleen from two IDDM patients at the onset of the disease. Within each pancreas, some islets with a few insulin-producing cells were present. Many islets lacked functioning β -cells in the absence of demonstrable insulinitis, whereas the other islets had an abundant CD4⁺ lymphocyte infiltrate. CD8-positive cells were also present, but in relatively smaller numbers (Fig. 1). Islets were separated from the exocrine pancreas by an automated method^{3,4} which provided, in both cases, more than 100,000 islets of more than 90% purity. When the TCR V β repertoire of the IIT cells of the first patient was analysed^{5,6} quantitatively, an exceptionally high level (>30%) of expression of the V β 7 family was found. In the spleen, the level of TCR V β 7 gene expression (~11%) approached that of V β 7 expression in peripheral blood lymphocytes (PBL) from non-diabetic individuals (8 $\pm$ 3.59%) (Fig. 2a-c). In contrast, the TCR V α repertoire⁷ among these IIT cells or splenocytes did not indicate an overexpansion of any V α family (not shown).

These results were confirmed by the study of a second, newly diagnosed IDDM patient. TCR expression on the IIT cells from this young boy showed a strong overexpression (~25%) of the V β 7 family as well. In the spleen, the TCR V β 7 gene was expressed at a level (~11%) closer to that of non-diabetic controls. To study this case, a second, larger set of TCR V β primers⁸ was used in parallel with Choi's set⁵ (Fig. 2g-i). Again, in this second patient, the V α repertoire was not skewed (not shown). We next examined the pattern of V β expression in PBL from randomly selected, non-diabetic individuals ($n=6$), following co-culture with the patient's islet cell membranes (Fig. 2). In all cases, we observed a consistent, several-fold increase in expression of V β 7-positive clones out of the 24 V β families tested⁸. Different TCR V β s, other than V β 7, were sporadically found

TABLE 1 Nucleotide sequence analysis

Source	V β	*	(N)D β (N)	J β
Patient 1 IIT	7.3	CASSQ	GITASY	1.2
	7.3	CASSQ	DKSGEVGE	1.4
	7.2	CASSQ	VHGQ	1.5
	7.2	CASSQ	VSRYS	1.6
	7.3	CASSQ	VSGN	2.1
	7.3	CASSQ	VSGN	2.1
	7.2	CASSQ	VHGN	2.1
	7.2	CASSQ	VAGAGT	2.2
	7.3	CASSQ	EGSGGPT	2.2
	7.3	CASSQ	VAGGPT	2.2
	7.2	CASSQ	DASGGQT	2.2
	7.2	CASSQ	DGGLY	2.7
	7.3	CASSQ	PTDGY	2.7
	7.3	CASSQ	RRGLADE	2.7
Patient 2 IIT	7.1	CASSQ	TGAWWA	1.1
	7.3	CASSQ	VQPRQGC	1.4
	7.2	CASSQ	DSGNKE	1.4
	7.2	CASSQ	STGTAN	1.4
	7.1	CASSQ	AMGEGAN	1.5
	7.3	CASSQ	LGDTL	2.1
	7.3	CASSQ	LHTSRV	2.1
	7.1	CASSQ	ILGND	2.1
	7.1	CASSQ	ASGN	2.1
	7.1	CASSQ	PGLAGPQGT	2.1
	7.1	CASSQ	GSPSFHN	2.1
	7.2	CASSQ	FGQGGPPAEF	2.1
	7.1	CASSQ	ATCAHST	2.1
	7.1	CASSQ	GAGVSSLA	2.1
	7.1	CASSQ	GSGSFH	2.1
	7.1	CASSQ	DLGSSQR	2.2
	7.2	CASSQ	SGQAGTG	2.2
	7.1	CASSQ	RRSPAG-	2.2
			GARE	
	7.1	CASSQ	SPKGTB	2.3
	7.1	CASSQ	DKGAA	2.3
	7.1	CASSQ	PGHAGLVT	2.3
	7.1	CASSQ	FPAGGLGD	2.6
	7.1	CASSQ	VTGEQ	2.7
	7.1	CASSQ	HKPGGQGT	2.7
	7.1	CASSQ	GFREGI	2.7
	7.1	CASSQ	EQAGGMY	2.7
	7.1	CASSQ	DLRGSTSY	2.7
	7.1	CASSQ	GSAGSY	2.7
	7.1	CASSQ	AGTGLG	2.7

Nucleotide sequence analysis of the TCR V β and CDR3 regions expressed in the T cells present in isolated pancreatic islets from the two diabetic patients studied. The one-letter notation has been used to show the deduced amino-acid sequences of the TCR V β and CDR3 regions expressed in the IIT cells from patients 1 and 2. The asterisk defines the amino acid in position 100 of the TCR β chain. V β 7 sequences from the first ($n=14$) and the second patient ($n=29$), respectively, are listed in order by the junctional (J β) regions. Anchored and nested PCRs were performed as described by Loh *et al.*¹⁰. Primers were selected according to Kohsaka *et al.*¹¹. Messenger RNA was extracted from 5×10^3 cryopreserved, unstimulated islets with oligo-dT cellulose (Fast Track; Invitrogen). Reverse transcription, RNA template digestion and purification of first strand DNA including dC-tailing were performed with 1/10 of the mRNA according to the manufacturer's suggestions (5' RACE System; GIBCO/BRL Life Technologies). First-round PCR consisted of 25 cycles after initial denaturation at 94 °C for 3 min; 94 °C for 20 s, 42 °C for 30 s, elongation at 72 °C for 45 s, followed by final extension at 72 °C for 10 min. Amplified products were size-selected (500–750 bp) on a 2% agarose gel, purified and 1/3 of the recovered material subjected to a second nested PCR. Nested C β 1- and C β 2-specific primers¹¹ at the 3' end, and the supplier's 5' universal amplification primers (UAP) were used in 25 cycles of PCR reaction: 94 °C for 20 s, 53 °C for 30 s and 72 °C for 45 s. TCR β -specific PCR-amplified material was once more size-selected, purified and cloned into Bluescript SK- (Stratagene). Out of ~600 recombinant plaques, 23 hybridized strongly²⁰ with a radioactively labelled V β 7-specific oligonucleotide probe⁸, whereas, for example, only nine plaques were positive with the V β 2 probe⁸, one of the most frequently expressed families in the general population^{5,6,8}. In addition, minipreps were prepared according to Maniatis *et al.*²¹ for nucleotide sequencing, revealing that 34% of the cDNAs were not TCR transcripts, 37% contained only partially rearranged (DJC) TCR β inserts²², and 14% did not contain V β segments long enough to be correctly attributed to any family. Of the remaining 15% comprising correctly rearranged V β cDNAs, 30% were V β 7.

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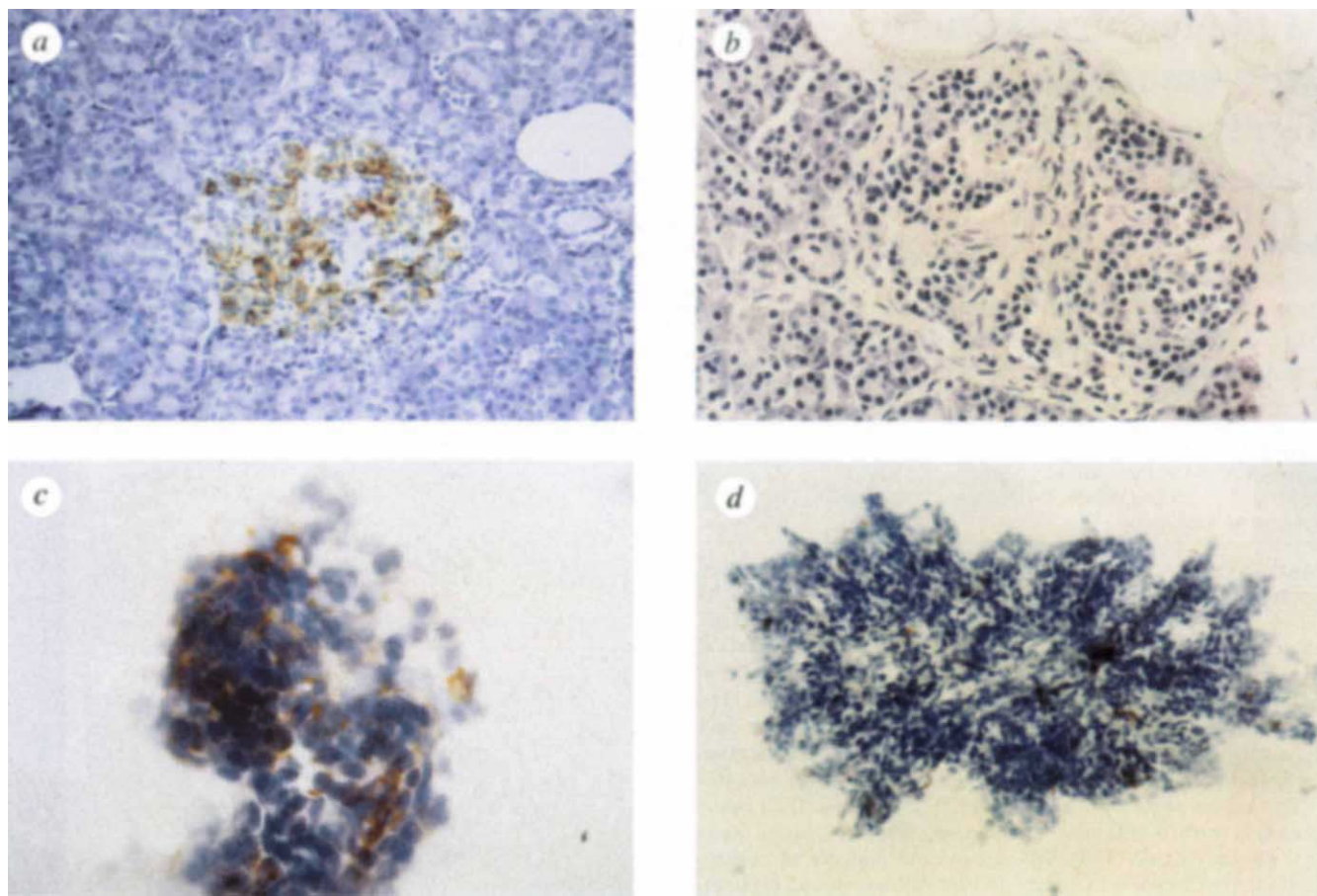


FIG. 1 In the first patient's pancreas, a few insulin-secreting β -cells were present as revealed by immunoperoxidase staining using an anti-insulin mAb (BioGenex Labs) (a). Some islets had a fibrotic ribbon-like appearance, and were partially or totally devoid of insulin-secreting cells (b). T-cell infiltrate (brown) was abundantly CD4⁺ (c) and scarcely CD8⁺ (d), as recognized by specific mAbs (Biosource Int.) in frozen preparations of isolated pancreatic islets. In May 1992, a 12-year-old boy was first seen with a chief complaint of vomiting and abdominal pain persisting from the previous night. He was somnolent but responsive, was oriented to persons, place and time, and had Kussmaul respirations. Serum glucose level was 81.2 mg dl⁻¹, acetone positive, and urinalysis revealed 4+ glucose, 3+ ketones and 1+ blood with a trace of protein. Venous blood gas analysis showed a pH of 6.94. It was learned that he had polyuria and polydipsia of approximately one month's duration. Type I diabetes mellitus, complicated with diabetic ketoacidosis, was diagnosed and fluid rehydration and insulin therapies promptly initiated. However, after ~6 h of treatment he fell asleep, and was unarousable.

Shortly thereafter, the patient underwent a cardiovascular and respiratory arrest secondary to severe brain swelling with cerebellar tonsillar herniation. Due to his critical condition, the boy was transferred for further care to the Children's Hospital of Pittsburgh, where he arrived brain dead. The history of the second, 11-year-old boy is strikingly similar to this one, except that the institutions from which these two patients came are geographically separated. Once an informed consent from the parents was obtained, the pancreas was processed immediately after the autopsy. The serological HLA typing of the first patient was A2, 28; B44, 60; Cw3; DR4, 11; DQ3, but the molecular typing^{23,24} revealed heterozygosity at both DQA1 (*0501, *0301) and DQB1 (*0301, *0302) loci. As the typing of the second patient was A1, 24; B27, 44; Cw1; DRB1*0101, *0301; DQA1*0101, *0501; and DQB1*0201, *0501, the possibility of forming 'diabetogenic' (α Arg52- β non-Asp57) DQ heterodimers²⁵ existed in both cases. Islet cell antibodies were greater than 20 JDF units in the first patient's serum, but less than 20 in that of the second.

FIG. 2 Autoradiographs of the TCR V β chain gene expression analysis in IIT cells (a) and spleen cells (b) from the first patient. In c, percentages of V β expression derived by scanning (Molecular Dynamics PhosphorImager SI) the gel shown in a (black bars) and in b (white bars) are compared with the percentages found in the PBL from six non-diabetic donors (cross-hatched with s.d.). These data were obtained using the primer set of Choi *et al.*⁵ and are described using their terminology. The upper panels of each autoradiograph show TCR V β families from 1 to 10, and the lower panels from 11 to 20. In the second column, an example of the percentages of V β expression in the T cells from PBL of six unrelated, non-diabetic individuals tested before (d) and after (e) exposure to the patient's islet cell membrane proteins (IMP No. 1). The white bars in the graph (f) represent the values obtained by scanning the gel shown in d, while the cross-hatched bars are from the gel shown in e. Black bars represent the pattern of V β expression after the first stimulation (this gel is not shown), whereas cross-hatched bars represent expression values after three cycles of stimulation. The first two

cycles of stimulation were set-up with IMP No. 1, and for the third cycle IMP No. 2 were used instead. These data were obtained using the primer set of Hand *et al.*⁶ and are reported with the appropriate family names. In the third column: the TCR V β analysis of IIT cells (g) and spleen cells (h) from the second patient. In i, we compared V β percentages from the gel shown in g (black bars) and the gel shown in h (white bars) with V β percentages in PBL from six non-diabetic donors (cross-hatched bars with s.d.). In the fourth column: the V β percentages in the T cells from the PBL of one of the three unrelated, non-diabetic individuals tested before (j) and after (k) exposure to the IMP No. 2. The white bars in the graph (l) represent the values from the gel in j and the black bars the values from the gel in k, and the cross-hatched bars represent the values obtained after three cycles of stimulation (this gel is not shown). Amplification of family 24 was detected by scanning the gel shown in k but is not visible in this photograph of the gel. IMP No. 1 and IMP No. 2 were prepared according to Gorga *et al.*²⁶

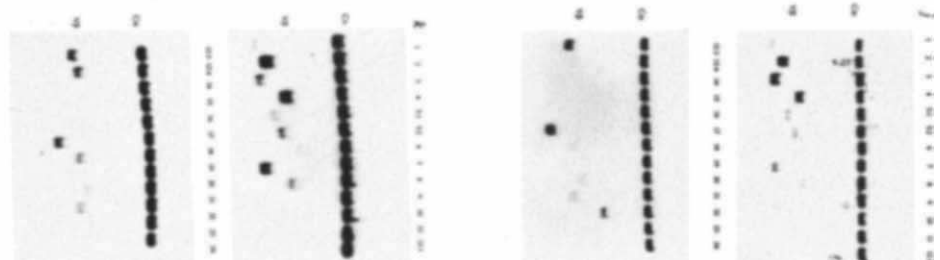
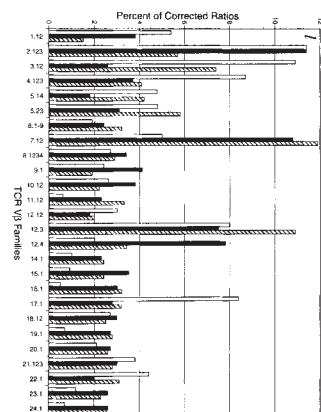
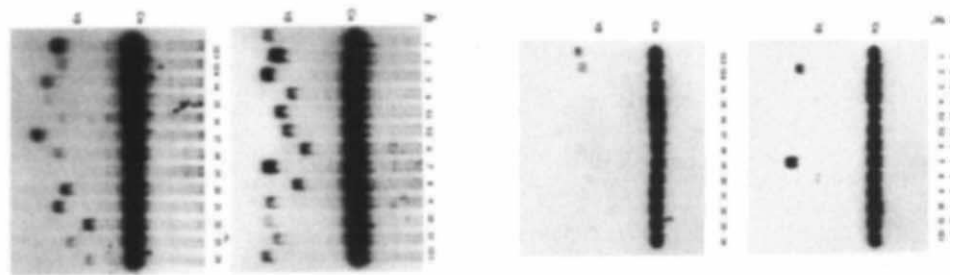
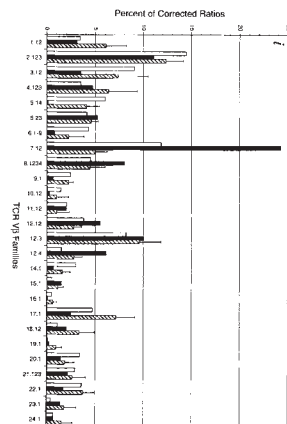
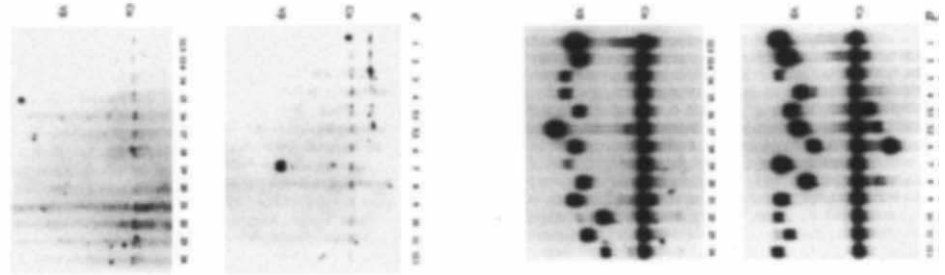
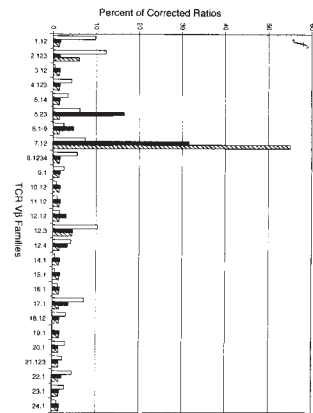
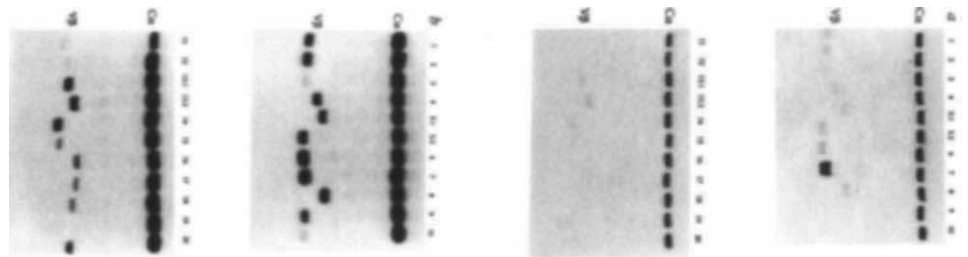
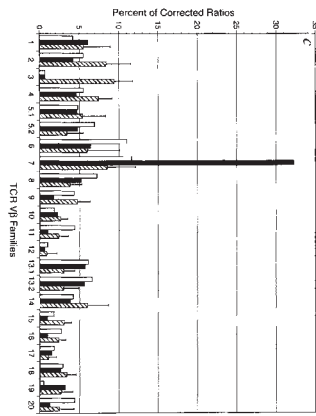
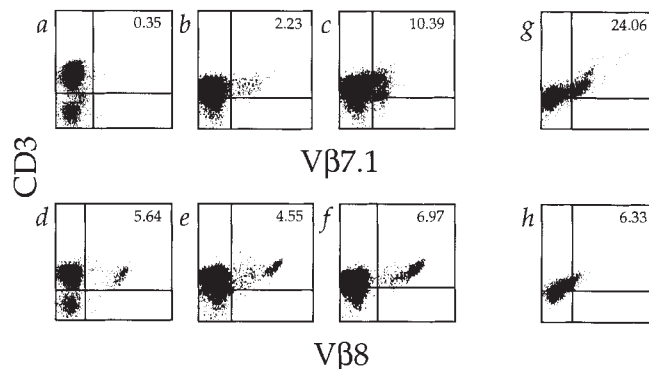


FIG. 3 Fluorescent flow cytometric analysis of PBL from non-diabetic donors before and after stimulation with patient's islet cell membranes. The expression of the subfamily $V\beta$ 7.1, as assayed by reactivity with mAb 3C5 (ref. 9), varied from 0.5 to 1% of $CD3^+$ peripheral T cells before stimulation (a), as originally reported⁹, to 3–5% after the first stimulation with $1 \mu\text{g ml}^{-1}$ islet cell membrane protein per 1×10^6 lymphocytes (b), and after a second cycle of antigen exposure, to more than 10% (c). Isotype control IgG2b mAbs (anti- $V\beta$ 8 and anti- $V\beta$ 12; T-cell Sciences) detected 4–7% of the $CD3^+$ T cells either before (d) or after the first (e) and the second (f) stimulation. Detection of $V\beta$ 7⁺ cells was obtained using $1 \mu\text{g}$ per 1×10^6 cells of a fluorescein isothiocyanate (FITC) labelled goat anti-mouse IgG (Fc) antibody (Caltag Laboratories, Inc.), while $CD3^+$ cells were visualized using a mAb directly conjugated with phycoerythrin. Exposure to non-diabetic, allogeneic islet membranes, and mitogenic stimuli (for example, anti- $CD3$ mAb) did not influence $V\beta$ 7.1 expression nor the expression of the isotype controls, β 8 and $V\beta$ 12 (not shown). The mAb 3C5 (anti- $V\beta$ 7.1) detected ~25% of the $CD3^+$ lymphocytes isolated from the pancreatic islets of our second patient (g), whereas mAb anti- $V\beta$ 8 detected ~6% of $CD3^+$ cells from the same preparation (h). Following a three-day exposure to anti- $CD3$ antibody, the islets were reduced to a single cell suspension by repetitive passages through a 30G needle, and the T cells enriched by



repeated exposures to magnetic beads (DYNAL AS) armed with an antibody specific for the human α -chain of the interleukin-2 receptor (IL-2R) (anti- $CD25$; BioSource Int.). The recovered cells were then cultured for 24 h to facilitate their separation from the beads before their use in two-colour FACS analysis.

to be overexpressed in some individuals following initial stimulation (for example, $V\beta$ 12.4 in Fig. 2k); however, this overexpression of non- $V\beta$ 7 clones disappeared with prolonged stimulation, whereas $V\beta$ 7 overexpression persisted (Fig. 2l). This preferential expansion was sustained even when islet cell membranes from the second patient were substituted with those of the first and vice versa (Fig. 2f). Allogeneic major histocompatibility complex (MHC)-related and MHC-unrelated islet membranes from non-diabetic individuals failed to affect $V\beta$ 7 expression under the same experimental conditions, as did conventional mitogenic stimuli (that is, interleukin-2 and anti- $CD3$) antibodies or phytohaemagglutinin. The $V\alpha$ repertoire remained unskewed in all the experiments (not shown). $V\beta$ 7-specific selection was also confirmed by fluorescence-activated cell sorting (FACS) analysis (Fig. 3a–f). The percentage of $CD3^+$ cells positively labelled with a mouse anti- $V\beta$ 7.1 monoclonal antibody (mAb)⁹ increased 5–10-fold when the same PBL were compared before and after exposure to patient's islet cell membranes. The percentage of $CD3^+$ cells expressing other $V\beta$ families (for example, $V\beta$ 8 and $V\beta$ 12) was not affected by stimulation with the same antigen. Flow analysis using the same antibodies (for example, anti- $V\beta$ 7.1 and anti- $V\beta$ 8) also confirmed the high percentage (24.06% compared with 6.33%, respectively) of $V\beta$ 7.1-positive T cells in the islets of the IDDM patients, before any specific stimulations (Fig. 3g and h).

T-cell clones grown from the isolated islets of both patients were characterized as $V\beta$ 7⁺ and $CD4^+$, by both FACS and polymerase chain reaction (PCR) analyses. No $CD8^+$ clones were isolated, perhaps because of the limited number of cell clones generated ($n=36$ and 25, respectively) (not shown). Finally, the TCR repertoire of the few T cells present in the isolated islets of two additional diabetic patients who died 25 and 29 years, respectively, after IDDM onset did show levels of expression of the $V\beta$ 7 family lower than normal values ($\leq 3\%$). Furthermore, normal percentages of $V\beta$ 7⁺ cells were found among the T cells present in the islets isolated from the pancreas of three non-diabetic organ donors (not shown). These experiments indicate that $V\beta$ 7⁺ cells do not preferentially home to human pancreatic islets in healthy individuals, nor is the $V\beta$ 7 preferential expansion a feature of long-term disease.

Anchored PCR^{10,11} experiments, performed on unstimulated IIT cells, further confirmed these results and demonstrated the variability in complementarity-determining region 3 (CDR3) among $V\beta$ 7⁺ clones and the polyclonal expansion of these $V\beta$ 7⁺ cells (Table 1). The high percentage of TCR $V\beta$ 7⁺ among the T cells present in the diabetic islets is consistent with a single-antigen-driven expansion of an unselected pool. The extensive junctional diversity of these $V\beta$ 7⁺ T-cell clones, however,

together with the results from the selection assays, points to the involvement of a superantigen rather than a conventional antigen in the aetiology of IDDM. Superantigens are unique products of ubiquitous bacteria and viruses¹. In the light of historical findings¹², viruses might be the modifying agent responsible for superantigen presence on the diabetic islets. Superantigens can also be the product of endogenous or integrated retroviral genes¹³, and in consideration of the role of breast compared with formula feeding in the risk for IDDM¹⁴, an example of these superantigens is the milk-borne, infectious, mouse mammary tumour virus (MMTV)¹⁵. MMTV is normally transferred with the milk from the C3H/HeJ mother to the pups during nursing, and is responsible for superantigen-induced clonal deletion of $V\beta$ 14-expressing T cells in the offspring¹⁶.

Thus, we can hypothesize that if the first exposure to the superantigen is at a very early age, potentially autoreactive T-cell clones are inactivated. The silencing of such 'dangerous' T-cell clones may then protect against the development of IDDM. If, instead, the first exposure to this superantigen is years after birth, many different T-cell clones expressing the same TCR $V\beta$ will become simultaneously activated. In a genetically predisposed individual, some of these T cells are able to initiate the process that eventually results in the destruction of the β -cells of the pancreas. Cytoplasmic antigens (such as ICA 69 and GAD₆₅; refs 14, 17) which become accessible to T and B lymphocytes as a consequence of initial islet cell damage would, in turn, maintain the stimulation of self-antigen-selected T-cell clones and self-antigen-specific B-cell responses, thus augmenting the destruction of pancreatic β -cells^{18,19}. Continuing studies are aimed at determining whether the superantigen (and perhaps the responsible virus) is actually present on the β -cells of the pancreas or was brought into the islets by infected lymphocytes. Exposure to superantigens as the triggering event in the pathogenesis of IDDM is a compelling novel hypothesis in which the diverse observations made in the fields of immunology, virology, genetics and epidemiology coalesce. □

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Calcium signalling in T cells stimulated by a cyclophilin B-binding protein

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THE immunosuppressant drug cyclosporin A blocks a calcium-dependent signal from the T-cell receptor (TCR) that normally leads to T-cell activation^{1–3}. When bound to cyclophilin, cyclosporin A binds and inactivates the key signalling intermediate calcineurin^{4–6}. To identify potential cellular homologues of cyclosporin A that might regulate calcium signalling, we have cloned human genes encoding cyclophilin B-binding-proteins using the yeast two-hybrid system^{7,8}. One gene product, when overexpressed in Jurkat T cells, specifically induced transcription from the interleukin-2 enhancer, by activating the T-cell-specific transcription factors NF-AT and NF-IL2A. This protein, termed calcium-signal modulating cyclophilin ligand (CAML), acts downstream of the TCR and upstream of calcineurin by causing an influx of calcium. CAML appears to be a new participant in the calcium-signal transduction pathway, implicating cyclophilin B in calcium signalling, even in the absence of cyclosporin.

We have isolated 10 plasmids encoding putative cyclophilin B-binding proteins from a human lymphocyte complementary DNA library using the yeast two-hybrid system^{8,9}. To identify a possible role in signalling, we investigated whether enforced

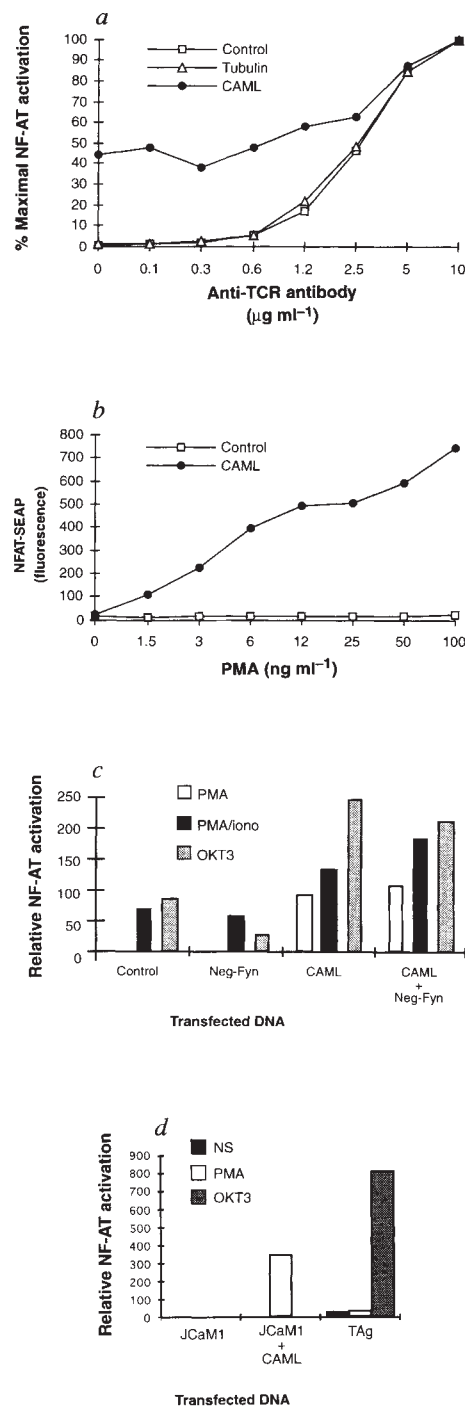
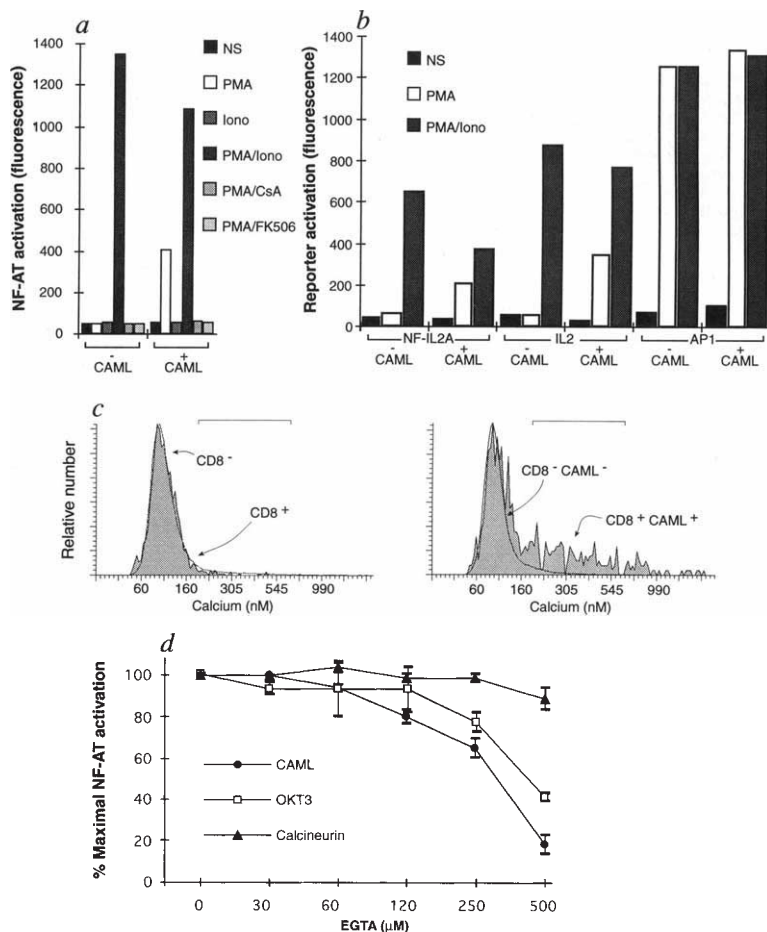


FIG. 1 Activation of transcription by CAML overexpression in T cells. **a**, CAML transfection replaces the TCR-stimulation requirement for NF-AT activation. TAG Jurkat cells co-transfected with the NFAT-secreting alkaline phosphatase (SEAP) reporter SXNFAT²³ and expression plasmids (based on pBJ5 (ref. 24)) encoding CAML (circles), tubulin (triangles), or nothing (squares) were stimulated with 25 ng ml^{-1} phorbol ester (PMA) and the indicated amounts of OKT3 (anti-TCR antibody). NFAT-specific transcription is expressed as a percentage of maximal induction by 10 $\mu\text{g ml}^{-1}$ OKT3. **b**, CAML induction of NF-AT activity requires PMA. The dependence of NF-AT activation on PMA concentration in CAML-overexpressing (circles) and control (squares) Jurkat cells

is shown. **c**, The block in T-cell activation induced by a dominant-negative p59^{fyn} is bypassed by CAML overexpression. TAG Jurkat cells were transiently transfected with NFAT-SEAP with or without pBJ-CAML and/or p59^{fyn}, a plasmid encoding a kinase-negative mutant form of p59^{fyn}. Cells were then treated as indicated, and NFAT-SEAP measured. To control for transfection efficiency, a constitutive promoter driving expression of luciferase (EF-Luc) was included. Phosphatase normalized to luciferase expression is indicated. **d**, CAML overexpression plus PMA activates NF-AT in spite of lack of Lck. JCaM1 (Lck-negative Jurkat cells) were transiently transfected with NFAT-luciferase reporter plasmid and pBJ5 (left) or pBJ-CAML (middle). TAG Jurkat cells were transfected with NFAT-luciferase and pBJ5. After 24 h cells were stimulated with PMA or OKT3 TCR antibody for 6 h and NFAT-specific luciferase was determined. RSV-SEAP reporter was co-transfected to normalize for transfection efficiency. METHODS. Transfections, drug treatments, and secreted phosphatase measurements were done as in ref. 23.

FIG. 2 CAML overexpression specifically activates calcium signal-dependent transcription factors by elevating intracellular calcium. **a**, CAML action is blocked by CsA or FK506. Tag Jurkat cells were co-transfected with NFAT-SEAP reporter plasmid and pBJ5 (left) or pBJ-CAML (right). After 24 h, cells were treated with the indicated combinations of drugs ([CsA] at 100 ng ml^{-1} , [FK506] at 500 pg ml^{-1}) for 20 h and NFAT-specific transcription determined.

b, CAML action is specific for calcium-dependent transcription factors. Induction of SEAP-reporter plasmids containing control enhancer sequences specific for NF-IL2A, AP-1 (from the metallothionein gene), or the entire IL-2 enhancer^{5,23} were tested as described in Fig. 1. Specific induction due to CAML is seen in PMA treated cells transfected with NF-IL2A or IL-2 reporter plasmids (open boxes). **c**, Intracellular calcium was measured by fluorescence-activated cell sorter described previously as in refs 25, 26, with CAML-overexpressing cells detected using murine CD8 α as transfection marker as in ref. 27. Control experiments were done to ensure that CD8 α overexpression did not inhibit or stimulate T-cell activation by CAML (data not shown). Cells were warmed to 37°C and treated with 25 ng ml^{-1} PMA immediately prior to analysis to simulate conditions shown to activate NF-AT in Fig. 1. Similar results were obtained without PMA. The top 50% of the CD8 α -positive cells representing the transfected cells are shown by the shaded curves; unshaded lines represent untransfected cells in the same cultures. Brackets indicate the intracellular calcium level following ionomycin treatment at the end of the experiment. **d**, CAML activation of NF-AT requires extracellular calcium. NF-AT activation was measured in Jurkat cells overexpressing CAML (circles), nothing (squares), or a C-terminal truncated, calcium-independent calcineurin A subunit²⁰ following treatment with PMA (circles and triangles) or PMA plus OKT3 (squares), in the presence of the indicated levels of EGTA.



overexpression of the cDNA inserts (lacking GAL4 sequences) in T cells could modulate activation induced by TCR stimulation. As a measure of T-cell activation, we monitored the activity of NF-AT, a T-cell-specific, early activation transcription factor essential for inducible expression of interleukin-2 (IL-2; refs 3, 10–12). Overexpression of one cDNA clone (CAML) partially abolished the requirement for TCR cross-linking (Fig. 1a)—as judged by activation of NF-AT-specific transcription—when assayed in the presence of phorbol ester (phorbol myristate acetate; PMA) to provide a co-stimulatory signal. None of the nine other cyclophilin B-interacting clones tested (data not shown), nor a cyclophilin A-interacting clone (β -tubulin), had any effect in this assay. The degree of NF-AT activation by CAML varied from 20% to 125% of maximal (PMA + ionomycin) induction in multiple transfections, distinctly different from control transfections in which activation of NF-AT was not observed in cells stimulated by PMA alone.

Activation of NF-AT by CAML requires exogenous stimulation of protein kinase C (PKC) by PMA (Fig. 1b), unlike TCR-mediated activation, which is sufficient to activate both calcium and PKC signal transduction pathways (data not shown). This suggests that CAML produces its effect in the calcium pathway, downstream of the TCR and phospholipase C. We examined whether CAML activation could bypass a block in signalling through the TCR caused by a dominant-negative form of p59^{lyn} tyrosine kinase^{13–15}. Although kinase-negative p59^{lyn} effectively reduced signalling through the TCR by 69–81% in separate transfections, it had no effect on activation induced by CAML overexpression in the presence of PMA (Fig. 1c, open boxes). This suggests that CAML acts downstream of the TCR:lyn complex.

We tested CAML in JCaM1 cells, mutant Jurkat cells unable to respond to TCR stimulation because they lack functional Lck (ref. 16). CAML overexpression activated NF-AT in PMA-treated JCaM1 cells (Fig. 1d) in spite of the Lck signalling defect. Together these experiments indicate that CAML functions downstream of the cell-surface receptor-associated tyrosine kinases Fyn and Lck.

To localize the CAML activation step further, we examined the effect of the specific calcineurin inhibitors cyclosporin A (CsA) and FK506. Addition of immunosuppressive amounts of either drug completely abolished CAML-mediated activation (Fig. 2a). This indicates that CAML does not act directly upon NF-AT (by, for example, titrating away an NF-AT inhibitory subunit), but instead acts upstream of calcineurin. To assess the CAML effect on two other transcription factors that regulate the IL-2 gene¹⁷, we tested reporter constructs linked to NF-IL2A¹⁸, AP-1-binding sites, or the entire IL-2 enhancer region. CAML partly replaced the calcium influx requirement for both NF-IL2A and the entire IL-2 enhancer, in a fashion similar to its effect with NF-AT (Fig. 2b, open boxes). As previously reported¹², without CAML there is no detectable expression from NF-IL2A or the IL-2 enhancer in the absence of calcium ionophore. CAML overexpression did not affect the activity of the AP-1 site from the metallothionein gene, which is calcium-independent (Fig. 2b).

These data suggest that CAML overexpression activates calcineurin in T cells. Such activation could be caused indirectly by elevation of intracellular calcium or could be due to a direct effect of CAML on calcineurin. To distinguish between these hypotheses, intracellular calcium levels in CAML-overexpressing cells were analysed by flow cytometry. There was a significant

a

CGCCACTGCCACCCCTCCAGACTGTGGACGGGAGGATGGAGTCGATGGCCGTCGCTACC 60
 GACGGGGGGAGAGGGCGGGGTCAGGGTCTGTCGGCTTCCAGCGTGGG 120
 GCGGAGCTCGTCCGGAGAAAGCTGCTCATGAACCTGGAAACAGCGCATCAACGGATCATG 180
 GGCCTTCACAGGCGCGGGAGCGGGAAGAAGTCAAAACAAATCAAGCAGCAG 240
 GACAGTGAATAAATCAACTCCCTCAGCGTCCCTTCGCTTCAAGCAGAGTAGTGTGGGT 300
 GATTCTAGTACAGGAACAACACTGACAGCAGGGTGGTGGCCGAGGTAAAGGGGACC 360
 CACTGGGAGACAAATTTGGACTCGTTCATTAAACACCTGAGTGCAGTAGTGTGCAAC 420
 CTTGAGCTCCGGCAGCGGAGAGGGGACCTGACAGCGGACTCGTCCAGAGGGTTCC 480
 CGCCATGGCTTAGAGCAGTACCTTTCCAGATTGCAAGAGCAATGAAGCTAAGGAAACAG 540
 CTCATTATGAAAAACCCAGTCAAGAGGATGGAATACACAGAAATTTGACTCTTTT 600
 CGAATATTAGATTGGTGGGATGTGCTCTTCTGCTCTGAGTCAAGCTTTTGTGTGC 660
 AAATATTTTCCCATATTTGCTCCATTTCTTACTTTTACAACTTGCCTACATGGGATATAC 720
 AAATATTTTCCCAAGAGTGAAGAAGATAAAGACAACAGTACTAACAGCTGCACCTCTA 780
 TTGTCGGGAATTCCTGCCGAAGTGATAATCGATCAATGGATACCTATAGCAAAATGGGC 840
 GAAGTCTTACAGATCTCTGTGTCTACTTTTCACTTTTATCTTTTGTGATGAAGCTGCTT 900
 GATTATTGGGGCTCTGAAGTACCATTGAAGCTGTAGAAGTGAAGGAGAGCTTACGAA 960
 AAAAAATCTCTTTCTATTGTCAGTGTCTTAAAGGAGGCAATTTGGTTTACACCTTCATG 1020
 TAATTTCTTTACTTTAGGGGTGTAAAGCTACTTTATTAGATATAGAATGGCAGATTCTC 1080
 TGATTAAAGGGCTGAGTTGTATTATTACTGATATGAAGAATAGTAGTACCAATGTGATC 1140
 TAATTGATTTTCTTGTATATCAGAAATCTCTATCTGTACCTTTCTCTAACTTCTCAGA 1200
 TTTGTAATTCCTTTCTGAGGAGCTGAGCTAGTGTCTTTAGGAGAACAGATAAATGTGGT 1260
 CTCAGCAGCGCTAGAGACTGCTTCTGTGTGTGTGTCATTCTGCTCTGAGAAATGAAGT 1320
 CATCTGAAAAATAAAATGCAGAAACCCAAAAAATTTTTTTTTTTTTTTTTTTTTTTT 1380
 AAAAAAAAAA 1391

b

MESMAVATDGGGERPGVPAGSGLSASQRRARLRRRLKLMNS 40
 EQRINRIMGFHRPGSGAEESQTKSKQDDKLNLSVPS 80
 VSKRVVLGDSVSTGTTDQGGVAEVKGTQLGDKLDSFIKP 120
 PECSSDVNLELRQRNRGDLTADSVQGRSRHGLEQYLSRFE 160
 EAMKLRKQLISEKPSQEDGNTTEEFDSFRIFRLVGCALLA 200
 LGVRAFVKYLSIFAPFLTLOLAYMGLYKYPKSEKKIKT 240
 TVLTAALLLSGIPAEVINRSMDTYSKMGEVFTDLCVYFFT 280
 FIFCHELLDYWGSEVP 296

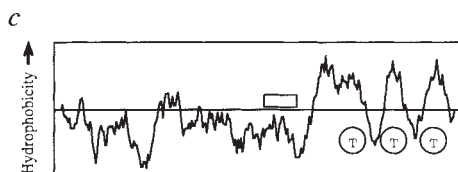


FIG. 3 DNA and protein sequences of CAML cDNA. **a**, DNA sequence of a full-length CAML cDNA isolated from a human fetal brain cDNA library probed using the yeast two-hybrid clone. Nucleotides 1 to 958 were present in the two-hybrid selection library insert. The initial ATG and final stop codon TGA are underlined. The first ATG is preceded by a consensus sequence for translation initiation²⁸, and is followed by an open reading frame of 888 bp. **b**, Predicted protein sequence of CAML. Three hydrophobic regions are underlined. *In vitro* transcription/translation of CAML produced a single polypeptide whose migration on an SDS-polyacrylamide gel was consistent with the predicted molecular mass of 33K (data not shown), in agreement with the DNA sequence determination. **c**, Kyte-Doolittle hydrophobicity plot of CAML protein. Regions predicted to be transmembrane domains²¹ are indicated by circled T's. The rectangle marks the stretch of amino acids (residues 153–178) predicted by Chou-Fasman and Robson-Garnier methods^{29,30} to be an α -helix.

increase in the baseline level of intracellular calcium in CAML-overexpressing cells as compared with control cells, indicating that CAML could activate calcineurin by causing calcium influx (Fig. 2c).

The dependence of CAML activation on extracellular calcium was examined by transient transfection of either CAML or a constitutively active, calcium-independent form of calcineurin A^{6,19,20}. Removal of extracellular calcium from the medium inhibited CAML activation of NF-AT, but had little effect on constitutive calcineurin activation (Fig. 2d). Thus, CAML overexpression activates the calcium-dependent pathway by causing calcium influx, rather than directly binding to and activating calcineurin.

The CAML cDNA contains a single reading frame predicted to encode a protein of 296 amino acids (Fig. 3). The specificity of CAML interaction with cyclophilin B was verified by a two-hybrid reverse-swap experiment⁸. In addition, CAML did not

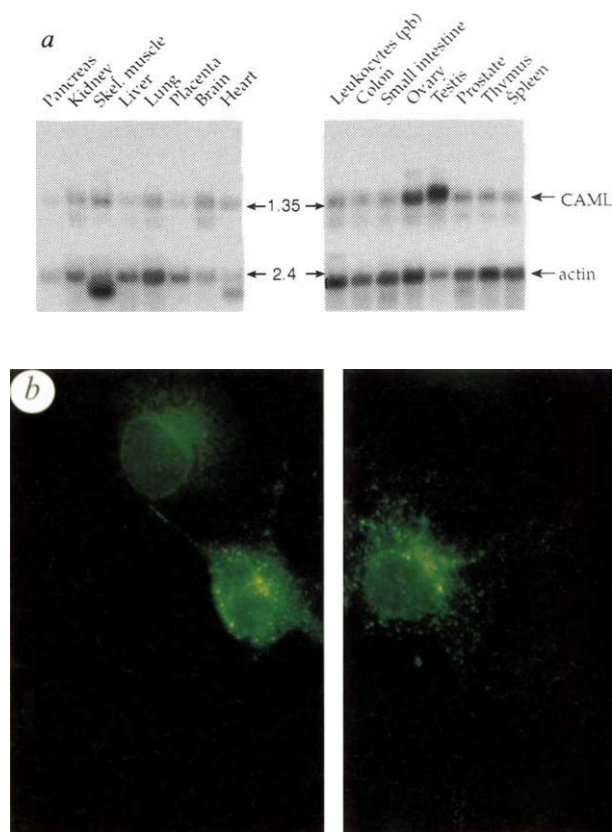


FIG. 4 **a**, Northern blot analysis of CAML mRNA expression in different tissues. **b**, Intracellular distribution of CAML.

METHODS. Nitrocellulose blots of poly(A)⁺ RNA from the indicated human tissues (Clontech) were hybridized with ³²P-labelled CAML DNA using standard techniques (top). The blots were subsequently stripped of DNA and re-probed with ³²P-labelled actin DNA (bottom). A DNA sequence encoding the FLAG epitope (DYKDDDDK) was fused to the N-terminus of the CAML open reading frame in the expression plasmid pBJ5. Addition of these residues did not impair the ability of CAML to activate NF-AT in Jurkat cells (data not shown). COS7 cells were transiently transfected with this plasmid by electroporation and grown in chamber slides for 24 h. Cells were fixed and stained as in ref. 23, except that the FLAG M2 monoclonal antibody (0.5 μ g ml⁻¹) was used. Several positive-staining cells are shown, surrounded by negative cells, which presumably did not take up DNA. No cells showed any specific staining in control transfections without FLAG-tagged CAML. Also, no staining was seen if TX-100 was omitted, implying CAML is intracellular.

interact with cyclophilins A or C, FKBP12, or calcineurin A (data not shown). Northern blot analysis revealed that the CAML message is expressed in all tissues (Fig. 4a), with the highest levels found in testis and brain. Three C-terminal hydrophobic regions of >20 residues are predicted to be transmembrane domains by the method of Sipos *et al.*²¹. CAML is therefore predicted to be an integral membrane protein with the majority of the polypeptide on the cytoplasmic face, consistent with its proposed role in calcium transport or regulation.

To identify the intracellular location of CAML, we performed immunofluorescence on COS cells transiently expressing an epitope-tagged derivative of CAML (Fig. 4b). CAML-specific staining was seen at vesicular-like structures scattered throughout the cytoplasm.

We identified CAML as a protein that binds to cyclophilin B in the two-hybrid system. Unlike CsA, the known inhibitory ligand of cyclophilin, CAML stimulates the calcium-dependent signalling pathway in T cells when overexpressed. We postulate that it normally participates in the regulated transmission of

the calcium-influx signal in lymphocytes and in other tissues, particularly brain and testis where it is more abundantly transcribed. CAML may bind cyclophilin B present at the calciosome²² (a subspecialized region of the endoplasmic reticulum involved in calcium homeostasis), and may regulate intracellular calcium release or generate the signal responsible for opening plasma membrane calcium channels. □

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CORRECTIONS

The genomic mutation rate for fitness in *Drosophila*

D. Houle, D. K. Hoffmaster, S. Assimacopoulos & B. Charlesworth

Nature **359**, 58–60 (1992)

It has come to our attention that an important stock used in this paper was probably contaminated during the experiments we reported. We accumulated spontaneous mutations in the virtual absence of natural selection on many copies of a single second chromosome of *Drosophila melanogaster*. To provide a control stock for these accumulation lines, we pooled six of the best performing lines, in two replicates, then maintained these stocks at a large population size to allow natural selection to purge any deleterious variation. In our paper we reported results from generation 44 of mutation accumulation. S. Nuzhdin and T. Mackay (personal communication) examined insertion sites of the transposable element 297 by *in situ* hybridization in the control stocks and found that about half of the insertion sites in both replicates were polymorphic. Our subsequent analyses of insertion sites of 297 and *roo* have confirmed this observation. This level of polymorphism is too high to be explained by transposition of these elements, but still far lower than that expected

in an outbred population. The results are most consistent with contamination of one of the six lines originally used to found both replicates of the control stock. We have also examined transposition sites in six mutation lines extracted at generation 62 of mutation accumulation for 297 and *roo*, and S. Nuzhdin examined an additional six lines for 297; none of these lines shows evidence for contamination (D. Houle *et al. Genetics*, in the press).

Consequently, we do not have a valid estimate of the rate of decline in mean fitness. Since our estimates of the genomic mutation rate and the average effect of a mutation are functions of this rate, our data cannot be used to make these estimates. On the other hand, our estimate of the increase in variance among replicate chromosomes is still valid. □

Mouse microcytic anaemia caused by a defect in the gene encoding the globin enhancer-binding protein NF-E2

Luanne L. Peters, Nancy C. Andrews, Eva M. Eicher, Mark B. Davidson, Stuart H. Orkin & Samuel E. Lux

Nature **362**, 768–770 (1992)

WE previously reported that the globin enhancer-binding protein NF-E2 was defective in the mouse hypochromic microcytic anaemia mutation *mk*. We based our conclusion upon the following. First, NF-E2 mapped to within 1 cM of the *mk* locus on mouse chromosome 15. Second, the *mk* phenotype, which includes decreased globin synthesis and defective iron uptake, made NF-E2 a logical candidate gene, especially once we established that NF-E2 was expressed in the proximal intestine. Third, the *mk* NF-E2 allele had a single base-pair difference at nucleotide 851 (T → C), predicting a valine-to-alanine substitution at amino acid 173, which was not present in DNA from its normal inbred strain of origin, DBA/2J (the *mk* mutation originally arose on C57BL/6J × DBA/2J F₂ mice; by Southern blot analysis, we determined that *mk* carried the DBA/2J NF-E2 gene). Since publication, however, S.-J. Lu, S. Rowan, M. R. Bani and B.-D. Yaacov (*Proc. natn. Acad. Sci. U.S.A.*, in the press) have shown, and we have confirmed, that normal inbred BALB/c mice also have C at position 851 of the NF-E2 gene, suggesting that the valine-to-alanine change is polymorphic in nature and does not represent the primary gene defect producing the *mk* mutation. We conclude that NF-E2 is closely linked to *mk* on mouse chromosome 15 but must be further evaluated in regards to its role, if any, in iron metabolism and in producing the *mk* phenotype. □

ERRATUM

Correlated neuronal discharge rate and its implications for psychophysical performance

Ehud Zohary, Michael N. Shadlen & William T. Newsome

Nature **370**, 140–143 (1994)

THE ordinate axis in Fig. 3a of this Letter should run from 1.0 to 10, and not from 0.1 to 10 as published. □

Immunology update

Research tools for the immunologist include a new class of mitochondrion-selective fluorescent dyes, ELISAs for urokinase-type plasminogen activator and cathepsin D and a host of antibodies and reagents.

MitoTracker is a new class of **mitochondrion-selective fluorescent dyes** from Molecular Probes that, unlike conventional fluorescent stains that are washed out once the mitochondrial membrane potential is lost, are said to be well retained during cell fixation and permeabilization (*Reader Service No. 100*). MitoTracker derivatives of both tetramethylrosamine and dihydro-tetramethylrosamine are available. Also available are derivatives of X-rosamine and reduced X-rosamine for researchers

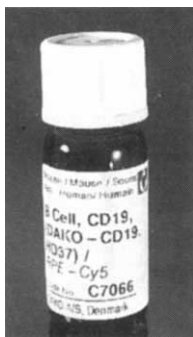


Staining using a mitochondrion-selective dye.

who need a longer-wavelength fluorescence emission for better resolution from fluorescein. The company says that the cell-permeant MitoTracker probes contain a thiol-reactive chloromethyl moiety. Once the probe accumulates in the mitochondria, it can react with accessible thiol groups on peptides and proteins to form an aldehyde-fixable conjugate. After fixation and permeabilization, cells can be double-labelled with an antibody directed against a mitochondrial protein or other cellular marker.

Flow cytometry

DAKO's new **RPE-Cy5 conjugated antibodies** have been optimized for use in flow cytometry (*Reader Service No. 101*). The fluorochrome is said to produce a sharp peak with an emission wavelength around 680 nm, so compensation with the PE signal is minimal. The conjugated antibodies are intended for analysing cell populations with low antigen expression. DAKO currently offers CD3, CD4, CD8, CD13 and CD19 conjugated with this fluorochrome.



DAKO's RPE-Cy5 conjugated antibodies.

AMAC announces the addition of **PE-Cy5 conjugated monoclonal antibodies** to its existing line of products for flow cytometry: CD3, CD4, CD19, CD34, CD45



Phycoerythrin-Cy5 conjugated antibodies for flow cytometry — see AMAC.

and IgG₁ phycoerythrin-Cy5 (*Reader Service No. 102*). AMAC's FITC, phycoerythrin and phycoerythrin-Cy5 are all excited at 488 nm, with FITC emitting at 525 nm, phycoerythrin at 575 nm and phycoerythrin-Cy5 at 670 nm. The majority of the photons emitted by the phycoerythrin in the tandem fluorochrome PE-Cy5 (>95 per cent) are captured by Cy5. This avoids light contamination in the PE detector by the PE-Cy5 dye spectrum. CD3, CD4, CD19, CD34, CD45 and IgG₁ phycoerythrin-Cy5 are available in liquid form.

PharMingen has introduced new **monoclonal antibodies against mouse and human Fas antigen** for studies in apoptosis (*Reader Service No. 103*). Suggested applications for the antibodies include flow cytometric analysis and induction of apoptosis *in vitro* via antibody-antigen interaction. New products for cell cycle analysis include antibodies against p21 (also known as Sd1, Waf1, Cip1 and Pic1) and p16, both of which are inhibitors of cyclin-cdk protein kinases and have been implicated in tumour suppression.

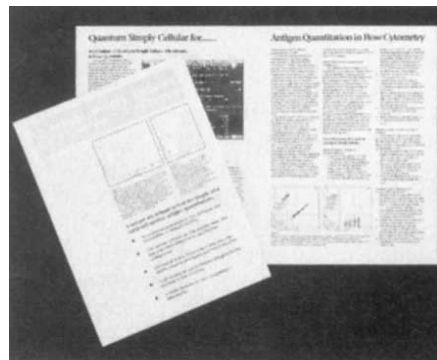
Becton Dickinson Immunocytometry Systems introduces **TriTEST reagents for multi-colour analysis** (*Reader Service No. 104*). The first in a series of products for flow



TriTEST reagents for flow cytometry.

cytometry, TriTEST reagents are a family of reagents that are designed to offer a three-step preparation process and to minimize hands-on work and tube handling. They combine a no-wash format with fluorescence-triggering and gating technology. The TriTEST reagents that are available immediately include IgG₁/IgG₂/CD45, CD3/4/45, CD3/19/45, CD3/16+56/45 and CD4/8/3. The new reagents are complemented by the company's LYSYS II (HP platform) and CELLQUEST software (FACStation), designed to simplify three-colour data acquisition and analysis in flow cytometry applications. New Attractors software (FACStation) adjusts automatically when cell populations shift, and, if time is an issue, the software can analyse batches of data at high speeds.

Quantum Simply Cellular is a **microbead system for the quantitation of cell-surface antigens** in flow cytometry that can be obtained from Sigma Immunochemicals (*Reader Service No. 105*). It consists of a mixture of four uniform microbead popula-

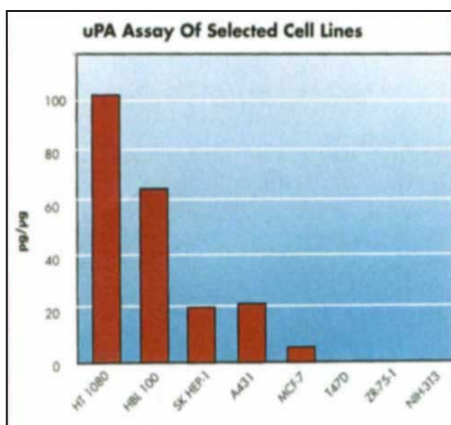


Cell-surface antigen quantitation protocol.

tions which differ by their incremental capacities to bind mouse immunoglobulin. The level of antigen expressed is measured in antibody binding capacity units. The microbeads are manufactured to be used with any mouse reagents in direct or indirect labelling protocols. They are labelled with the same antibody reagent(s) used for the cells. This system is not restricted to the detection of a specific antigen or fluorochrome. QuickCal is the data analysis software program provided that accommodates data generated by any type of flow cytometer (DOS or Winlist versions available).

Assays/kits

Oncogene Science introduces two new **quantitative immunoassays**, cathepsin D ELISA and urokinase-type plasminogen (uPA)



Urokinase-type plasminogen activator assay available from Oncogene Science.

ELISA (Reader Service No. 106). The former measures both the pro- and mature forms in human tumour cytosols and extracts, as well as cell culture-derived samples. The uPA ELISA is able to detect uPA bound to its receptor or PAI-1 bound uPA in tumour and serum cell culture derived samples.

Promega's **ELISA for the detection of biologically active TGF β_1** (transforming growth factor β_1) uses an antibody 'sandwich' format (Reader Service No. 107). The stated minimum level of detection of biologically active TGF β_1 is 25 pg ml $^{-1}$. The system contains sufficient reagents for 400 determinations (five 96-well ELISAs), plates not included. A **TGF β_1 western system** is also available which is designed for the detection of biologically active TGF β_1 with no cross-reactivity (of up to 10 ng) to TGF β_2 and TGF β_3 in a western or dot-blot format. Typically, a minimum of 300 pg of TGF β_1 can be detected by this method within 5 h, following transfer of samples onto nitrocellulose or PVDF membranes. Sufficient reagents are provided to perform 10 western blots or 960 dot-blot determinations. Completing the line-up — TGF β_1 , human, natural and anti-TGF β_1 pAb for ELISA detection and quantitation, western and dot-blot analysis, immunohistochemistry, immunoprecipitation and biological neutralization assays.

IBL has developed a direct ^{125}I -radioimmunoassay for the quantitative determination of melatonin in serum, plasma or saliva (Reader Service No. 108). No extraction of melatonin from serum and plasma is required because the binding proteins are degraded enzymatically before carrying out the RIA. The stated sensitivity of the assay is said to be 2.5 pg ml $^{-1}$ for serum and plasma and 1 pg ml $^{-1}$ for saliva. Also available, an enzyme immunoassay for the direct determination of melatonin-6-sulphate in urine.

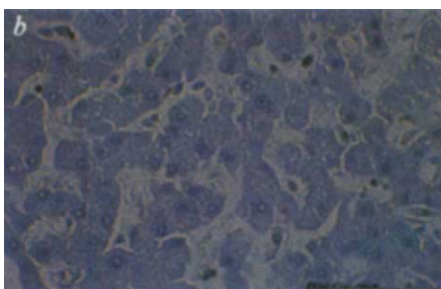
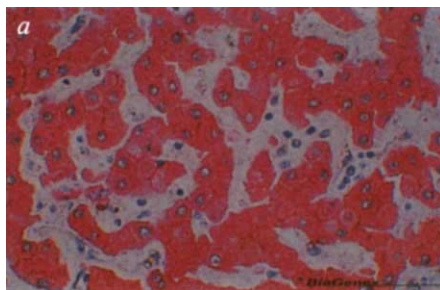
Now available from Accurate Chemical & Scientific, **ELISA kits for the estimation of fibrinogen/fibrin split products** generated by digestion with thrombin or

plasmin (Reader Service No. 109). Assays use peroxidase-labelled monoclonal antibodies specific for fibrinopeptide A, fibrinogen peptide B β 1–42, fibrin peptide B β 15–42. The assays take about 90 min to complete and have a stated sensitivity in the pmol ml $^{-1}$ range.

A new **IsoStrip mouse monoclonal antibody isotyping kit** is available from Boehringer Mannheim (Reader Service No. 110). Whether you are interested in testing ascites, hybridoma culture supernatants or purified monoclonal antibodies, it is possible to determine the isotype of any mouse monoclonal antibody (IgG $_1$, IgG $_{2a}$, IgG $_{2b}$, IgG $_3$, IgM and IgA) and the Ig light chain composition (λ or κ). Isotyping strips and development tubes are provided for performing the test using the three-step method.

Reagents

The new Antigen Retrieval technique from BioGenex can be used to **rescue antigens from formalin-fixed, paraffin-embedded tissue** (Reader Service No. 111). Use of the



Formalin-fixed liver tissue stained with MAb to low-molecular weight cytokeratin using the SuperSensitive detection system and Fast Red chromogen with (top) and without Antigen Retrieval.

company's latest product, the nontoxic Antigen Retrieval Citra solution, is expected to help overcome false negative staining of overfixed tissue, expand the range of antibodies used in routinely processed tissue and increase the usefulness of archival material for retrospective studies. It is designed for use with a method that recovers antigenicity during the microwave heating of tissue sections. This family of products also includes Antigen Retrieval DeCal, a room temperature pretreatment developed to recover staining in acid-decalcified tissue embedded in paraffin or celloidin.

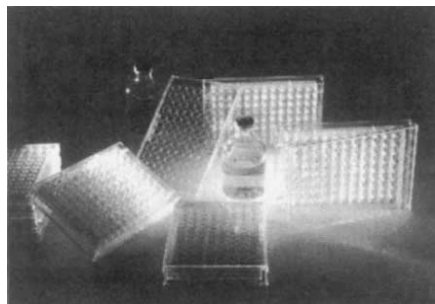
In association with Upstate Biotechnology, TCS Biologicals has introduced a **HeLa nuclear extract**, as well as a new range of **antibodies to transcription factors** (Reader Service No. 112). Polyclonal antibodies raised using purified recombinant antigen are available against human and yeast TFIID (the TATA binding protein), and also against TFIIB. Also available — polyclonal antibodies to TFIIE and TFIIF.

A range of **purified animal immunoglobulins** are now available from Harlan Bioproducts for Science (Reader Service No. 113). The line-up includes mouse, bovine, rabbit, rat and sheep IgG. These products have been purified through a combination of ion exchange, gel filtration, hydrophobic dye ligand affinity and immunoaffinity chromatography. All of the purified proteins are said to show a single precipitation line against the respective antisera, and all are supplied at a concentration of 10 mg ml $^{-1}$. The purified IgG fractions are supplied lyophilized and are shipped at ambient temperature.

Peptide Technologies is a supplier of purified peptides for use as immunogens. The company specializes in the supply of **synthetic antigens** such as MAPS peptides and 'peptomers' (Reader Service No. 114). For AIDS research, there is a highly water-soluble peptide substrate for the HIV-1 aspartyl protease, said to be useful for HPLC assays in drug screening. The peptide can be tritiated for high-sensitivity experiments. A new product under development is an analogue of phosphorylated tyrosine, Fmoc phosphonomethyl phenylalanine.

Detection/purification systems

New **streptavidin-coated microstrips** and breakable microstrips from Labsystems enable the biotin/streptavidin system to be used for binding DNA peptides, polysaccharides and other biotin conjugates to the polystyrene well surface (Reader Service No. 115). This system is designed to overcome the binding problems associated with steric hindrance or hydrophilic molecules, and is suitable for solid-phase DNA hybridization assays, in addition to immunoassays, receptor studies and other solid-phase assays. Each well of the microplate or strip is said to bind 6×10^{12} molecules of biotinylated oligonucleotide or approximately 10^{12}



Streptavidin-coated microplates.

molecules 100–500 base-pairs long DNA fragments. The full line includes clear plates, white microplates and strips for assays using luminescence markers and plates with a black pigment for fluorescence assays.

TissuGnost Uni-Pak is Merck's new universal **detection system based on the use of streptavidin/biotin**, which is designed for routine and research immunocytochemistry staining (*Reader Service No. 116*). Merck says the kit will detect all mouse monoclonals, all polyclonals from rabbit, goat, sheep and many more species. It is intended for both paraffin and cryostat sections. The kit comprises protein blocking reagent, secondary antibody (polyvalent, biotinylated), phosphate buffer concentrate and streptavidin-peroxidase concentrate. Sufficient reagents are included for up to 400 staining procedures.

Promega's new **EGGstract IgY purification system** provides a method for the isolation of IgY, the chicken IgG homologue, from egg yolks (*Reader Service No. 117*). The protocol and reagents that come with the kit allow the user to recover 55–80 mg of 75–90 per cent pure IgY per egg yolk in about an hour. Sufficient reagents are supplied with the kit for the purification of IgY from up to six eggs. IgY purified this way is suitable for use in standard immunological assays in conjunction with Promega's affinity-purified anti-chicken IgY, alkaline phosphatase conjugate; anti-chicken IgY, horseradish peroxidase conjugate; chicken IgY, control immunoglobulin; and with anti-chicken IgY, immobilized.

■ p53

Developed by Novocastra Laboratories and distributed by Vector Laboratories are five clones of **anti-human p53 antibody** sold in unconjugated, biotinylated and fluorescein-labelled form that are intended for use in immunohistochemistry, flow cytometry and western blots (*Reader Service No. 118*). A monoclonal and polyclonal antibody to the MDM2 protein (p53 binding protein) are also available. Four of the clones are mouse monoclonals: D07, 240, 1801 and BP (binding protein); one, p53-CM1, is a rabbit polyclonal. All are said to recognize the wild and mutant forms of human p53 protein, providing intense nuclear staining with no background. All can be used with frozen and paraffin-embedded sections with the exception of anti-p53-BP which is recommended for use with paraffin-embedded tissues only.

Biomedica's **p53 monoclonal antibody** recognizes an epitope in the N-terminus of both the mutant and wild type p53 protein that is localized in the cell nucleus, and can detect the various types of tumours that carry p53 mutations including those neoplasms located in the lung, testes, brain, bladder, breast, colon and stomach (*Reader Service No. 119*). The monoclonal antibody

to p53 can be used on formalin-fixed, paraffin-embedded tissue sections, and is compatible with Biomedica's immunostaining kits.

■ Catalogues

The 1994–95 Cedarlane Laboratories **catalogue of research and diagnostic reagents** for the immunologist is now available from VH Bio (*Reader Service No. 120*). New products include O-sialoglycoprotein endopeptidase from *Pasteurella haemolytica*, Lympholyte-Mammal for the isolation of lymphocytes from most mammalian species, Immunocolumns for the isolation of rat CD4 and CD8 cells, antisera to human coagulation proteins, paired antibodies for haemostasis research and additions to the anti-mouse cell-surface antigen range.

Now out — the new Calbiochem-Novabiochem **catalogue and peptide synthesis handbook** (*Reader Service No. 121*).



Peptide catalogue

Over 150 new products are featured, including resins for solid-phase synthesis, reagents for phosphopeptide synthesis, resins and reagents for the production of peptide libraries, immunogenic peptides and multiple antigen peptides.

■ Instrumentation

To achieve high-sample throughput for drug discovery and other microplate applications, Zymark is introducing MicroAssay technology for its Zymate systems. The latest **immunoassay system** uses multiple active automation modules for high speed, high capacity storage modules for unattended operation, and has the flexibility to handle multiple types of assays (*Reader Service No. 122*). Zymark plans shortly to introduce new assay systems specifically for cell-based and receptor binding assays, and for sample preparation and distribution applications. The system transfers 8, 12 or 96 wells at



High-throughput immunoassay set-up.

a time, stores up to 200 microplates and consumables, and prepares labile reagents online. Many of the modules can operate independently so multiple microplates can be processed simultaneously. The system can be used with most ultraviolet/visible, microplate readers, fluorometers and luminometers, and uses Windows-based software.

The new miniPERM system developed by Heraeus is a high-density **cell culture system** designed as an alternative to the ascites method of producing monoclonal antibodies (*Reader Service No. 123*). The system — a small bioreactor — consists of a nutrient storage chamber and a production chamber separated by a dialysis membrane which retains the cells and antibodies produced while allowing the exchange of nutrients and metabolic waste products. An additional silicone membrane is designed to ensure the rapid exchange of gases.

Endpoint and kinetic ELISAs, and scanning capabilities for determining agglutination patterns and cell counting are some of the design capabilities of Bio-Tek's new Ceres 900 **microplate reader** (*Reader Service No. 124*). Stated applications for the



Bio-Tek's Ceres 900 reader.

Ceres 900 include ELISAs, protein determinations, cytotoxicity studies, and studies of cell growth, MIC determinations and agglutination assays. Another model, the EL311 **scanning microplate reader**, interfaces directly with a CRT for display of absorbance data and reports. The Scan-A-Well feature provides up to three data points across each well for agglutination for MIC-type analysis. An internal barcode reader is an optional extra. The EL311 may be combined with the company's new Turbo Accelerator compact data analysis unit.

■ Cell biology research

The **chemokines**, a superfamily of secreted proinflammatory cytokines, are induced by inflammatory stimuli and are involved in regulating the selective migration and activation of leukocytes that mediate the inflammatory response. Superfamily members have been subdivided into two classes: the α or C-X-C class of chemokines which map to chromosome 4, and the β or C-C class, which map to chromosome 17. Now available from Bachem in the α class — rec IP-10 (human), rec IL-8 (human) and rec GRO/MGSA (human); those in the β class include rec MIP-1 α (human), rec MIP-1 β (human), rec MCP-1 (human) and rec RANTES (human) (*Reader Service No. 125*).

Biokine IL-10 is a new **cytokine test kit** available from T Cell Diagnostics for measuring soluble IL-10 levels in human serum, plasma and cell culture supernatant (*Reader*

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Reader Service No. 11

Service No. 126). The methodology is said to require only a 100 µl sample size and the whole assay incubation time is 2 h 45 min. The stated sensitivity of the assay is less than 10 pg ml⁻¹. Precoated 96-well strip plates and seven pre-calibrated standards (0–500 pg ml⁻¹) are provided with the kit.

Two new **inflammation/transplantation research antibodies** — polyclonal rabbit anti-human IL-10 and monoclonal mouse anti-human IL-8 — have been launched by Innogenetics and are available through New Brunswick Scientific (*Reader Service No. 127*). These two new products have particular relevance to research into bacterial sepsis, rheumatoid arthritis, psoriasis, allograft survival and T-cell mediated autoimmune disease such as type-1 diabetes and multiple sclerosis. The polyclonal rabbit anti-human IL-10 can be used in western blotting; mouse monoclonal anti-human IL-8 can be used to neutralize the bioactivity of human IL-8 and also as a 'capture' reagent for a 'sandwich' ELISA to quantify human IL-8.

Becton Dickinson Cellular Imaging Systems announces a new CAMFolio **monoclonal antibody for research into tumour biology**: anti-P-selectin (CD62P) (*Reader Service No. 128*). This monoclonal antibody functionally blocks platelet adherence to leukocytes, small cell lung cancer and myeloid cell lines (*Nature* **359**, 848–851; 1992 and *J. clin. Invest.* **92**, 804–813; 1993). Additional monoclonal antibodies include anti-integrin α5 (CD49e), anti-integrin αvβ5 I and anti-Lewis^x (CD15).

ET-18-OCH3 is Kamiya Biomedical's **synthetic analogue of lysophosphatidylcholine**, which is a member of a new class of membrane-active agents that show selective cytotoxic activity against neoplastic cells and virus-transformed cells (*Reader Service No. 129*). It should find application in cancer, signal transduction and cell biology studies. Kamiya Biomedical says that ET-18-OCH3 promotes rapid and selective apoptosis of human leukaemic cells, selectively inhibits phosphatidylinositol phospholipase C, inhibits protein kinase C and its phosphorylation of endogenous proteins in leukaemic cells, and is an inhibitor of Na, K-ATPase.

New from Transduction Laboratories — monoclonal and polyclonal **antibodies to the Raf (c-raf-1) kinase** (*Reader Service No. 130*). Both are intended for visualizing Raf in a variety of species by western blotting and are said to be capable of detecting the mobility shift of hyperphosphorylated Raf in cells stimulated with EGF. The monoclonal can also be used in immunoprecipitation and indirect immunofluorescence analysis. The company also supplies monoclonal **antibodies to several proteins involved in cell cycle regulation**: cdk4, cyclin D1, Rb and cdk2.

Three polyclonal **antibodies for the study of constitutive and inducible nitric oxide synthases (NOS)** are now available from Affinity BioReagents (*Reader Service No. 131*). The inducible NOS antibody can be used in western blot, immunocytochemical and immunoprecipitation experiments of cytokine-induced murine samples. Researchers can detect the constitutive forms of NOS with antibodies to endothelial cell NOS and brain NOS by western blot and immunohistochemistry. The monoclonal antibody to retinoid X receptor β (RXRβ) can be used in western blot, immunofluorescence, immunoprecipitation and gel supershift assays to detect human and mouse RXRβ. Additional steroid receptor antibodies are available against the retinoic acid, thyroid hormone, vitamin D, androgen, estrogen and progesterone receptors.

■ **Growth factor research**

R & D Systems offers both monoclonal and polyclonal **antibodies to human vascular endothelial growth factor (VEGF)**, in addition to the recombinant protein (*Reader Service No. 132*). Also referred to as vascular permeability factor or vasculotropin, VEGF has been found to be a potent angiogenesis inducer *in vivo* and may play a role in tumour formation. The recombinant protein is expressed in the insect cell line Sf21 and its ED₅₀, as determined by an umbilical vein endothelial cell bioassay, is said to be 1.0–3.0 ng ml⁻¹. The antibodies were raised against the 121 amino acid VEGF variant and will thus cross-react with all larger variants.

The angiogenic factor, **human vascular endothelial cell growth factor (VEGF)**, is now available from Biomedical Technologies (*Reader Service No. 133*). VEGF is a secreted homodimeric protein (38 kilodaltons) consisting of two covalently linked 165 amino acid chains. BTI's VEGF is produced using recombinant DNA techniques and is said to be >98 per cent pure (by SDS PAGE). ¹²⁵I VEGF is available on request.

Newly available from Bionostics, a **monoclonal antibody to human transforming growth factor β₁ (TGF β₁)** (*Reader Service No. 134*). Suggested applications include immunohistochemical staining, including paraffin-embedded tissues, and western blot analysis. Bionostics says that its product has been shown to be effective using bioassay techniques in the neutralization of TGF β₁ action on continuous cell lines. The monoclonal is supplied as a purified, lyophilized reagent. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. For more details on any of the products featured, fill in the reader service card bound inside the journal.